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THE AMERICAN
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THE REASON FOR THE SPECIFIC DYNAMIC ACTION OF PROTEIN

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From the Physiological Laboratory of the University of Illinois

Received for publication December 31, 1918

Lavoisier (1) showed that the ingestion of food increased oxidation in the body. Rubner (2) found that of the food materials, the ingestion of meat increased oxidation most, fat next and sugar least. Rubner designated the action of protein in increasing oxidation more than the other food materials as the *specific dynamic action of protein*. Several theories have been advanced in attempts to explain how the ingestion of food increases oxidation. The theory, or some modification of the theory of Voit, who claimed that the presence of food augmented the inherent power of the cells to metabolize, is the one that has met with most general acceptance. Lusk (3) originally attributed the increase in metabolism following the ingestion of meat, to the mass action of the amino acids acting as stimuli on the protoplasm of the cells. Later, (4) however he modified this explanation and attributed the increase in oxidation to the stimulating action of the organic acids resulting from the deamination of the amino acids, while Grafe (5) attributed the stimulating action of protein to the amino groups. We (6) found that the increase in oxidation produced by the ingestion of food, as well as by a variety of ways, was accompanied by an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide. We also found that meat, in keeping with its greater stimulating action on heat production, produced a greater increase in catalase than the other food materials, hence the conclusion was drawn that the increase in oxidation produced by the ingestion of food as in other ways, was due to an increase in catalase which was brought about

by the stimulation of the alimentary glands, particularly the liver, to an increased output of this enzyme.

Lusk found that the ingestion of amino acids, such as glycocoll and alanin, produced an increase in oxidation while glutamic acid did not. Grafe, on the contrary, found that the ingestion of glutamic acid as well as of glycocoll and alanin produced an increase in oxidation. We (6) found that the ingestion of glycocoll and alanin produced an increase in catalase and that glutamic acid did not. This fact was offered as an explanation for Lusk's observation that the ingestion of glycocoll and alanin increased oxidation while glutamic acid did not. Grafe (5) found that ingestion of asparagin, aspartic and succinic acids and acetamid produced an increase in oxidation. Recently Lusk (7) has repeated and confirmed his original work, extending it to include the effect of asparagin, aspartic and succinic acids, acetamid and ammonium citrate on heat production and found, contrary to Grafe, that these substances produced no increase in oxidation.

One of the objects of the present investigation was to determine if the ingestion of aspartic and succinic acids, acetamid and asparagin would or would not produce an increase in catalase. The animals used were dogs. After etherizing the animals and opening the abdominal wall each of these substances was introduced in about equal quantities into the stomach and upper part of the intestines. The amounts of the substances will be given under the individual experiments. The catalase in 0.5 cc. of blood taken from the portal and jugular veins and from the liver was determined before as well as at fixed intervals after the introduction of the materials. The determinations were made by adding 0.5 cc. of blood to 50 cc. of hydrogen peroxide in a bottle at approximately 22°C. and the amount of gas liberated in ten minutes was taken as a measure of the catalase content of the 0.5 cc. of blood.

In figure 1 are shown the effects of the introduction of glutamic, aspartic and succinic acids and of asparagin on catalase production. The acids were given in the form of sodium salts and in saturated solutions. The figures along the ordinate (0 to 100) indicate amounts of catalase measured in cubic centimeters of oxygen and those along the abscissae, time in minutes. The continuous line curves show amounts of catalase in the blood of the liver; the discontinuous line curves, amounts in the blood of the portal vein; and the dotted line curves, the amount of catalase in the blood of the jugular vein. It may be seen that the introduction of 14 grams per kilo of body weight of glutamic acid produced an increase in catalase as is indicated by the increase in the amount of

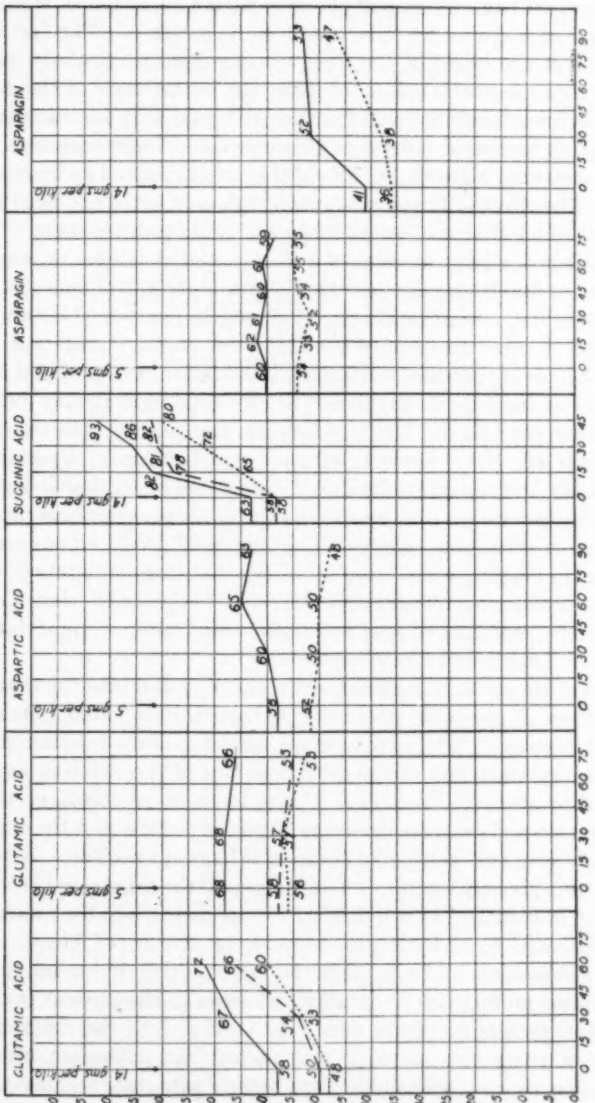
GLUTAMIC ACID
GLUTAMIC ACID
ASPARTIC ACID
SUCCINIC ACID
ASPARAGIN
ASPARAGIN
14 gms. per kilo
5 gms. per kilo
Time in minutes
Amounts of calories measured in cubic centimeters of oxygen

Fig. 1

oxygen liberated from hydrogen peroxide by 0.5 cc. of blood, while the introduction of 5 grams per kilo produced no increase. The fact that the catalase of the blood of the liver was increased particularly during the first fifteen minutes much more than that in the blood of the portal or jugular veins is taken to mean that the glutamic acid was stimulating the liver to an increased output of this enzyme. Under aspartic acid it may be seen that 5 grams per kilo of this acid produced no increase in catalase. The effect of larger amounts of this acid could not be studied owing to the fact that we had no more and could procure none. However, the effect of a large amount of a closely related substance, succinic acid, was tried and it may be seen that 14 grams per kilo of this acid produced a great increase in catalase. It should be mentioned in this connection that a small amount of succinic acid, about 1 gram per kilo, which Lusk found to produce no increase in oxidation, was used and found to produce no increase in catalase. Under asparagin it may be seen that 5 grams per kilo did not produce an increase in catalase while 14 grams per kilo did. Since small amounts of glutamic, aspartic and succinic acids and asparagin produced no increase in catalase in keeping with Lusk's observation that small amounts of these substances produce no increase in oxidation, whereas large amounts produced an increase in catalase, in keeping with Grafe's findings that large amounts of these substances produce an increase in oxidation, it is probable that the difference in the amounts of the substances used may account for the difference in Lusk's and Grafe's results.

In figure 2 are shown the effects of the introduction into the stomach and intestines of glycocoll, acetamid, sodium acetate, olein, potassium oleate and dextrose. The manner of introduction of the substances into the alimentary tract and of the determination of the catalase was the same as that already described. The amount of glycocoll, acetamid, sodium acetate and dextrose used was 20 grams per kilo and the amount of olein, glycerin and potassium oleate was 10 grams per kilo. All these materials were given in a saturated solution. All the substances produced an increase in catalase, as is indicated by the increase in the amount of oxygen liberated from hydrogen peroxide by 0.5 cc. of blood. In a previous publication we found that the introduction of sodium palmitate produced no increase in catalase, whereas in the present investigation we find potassium oleate does. In looking into the difference in solubility of these two soaps, it was found that potassium oleate is about twelve times more soluble than sodium palmitate and hence was absorbed, presumably more rapidly and more extensively, than sodium

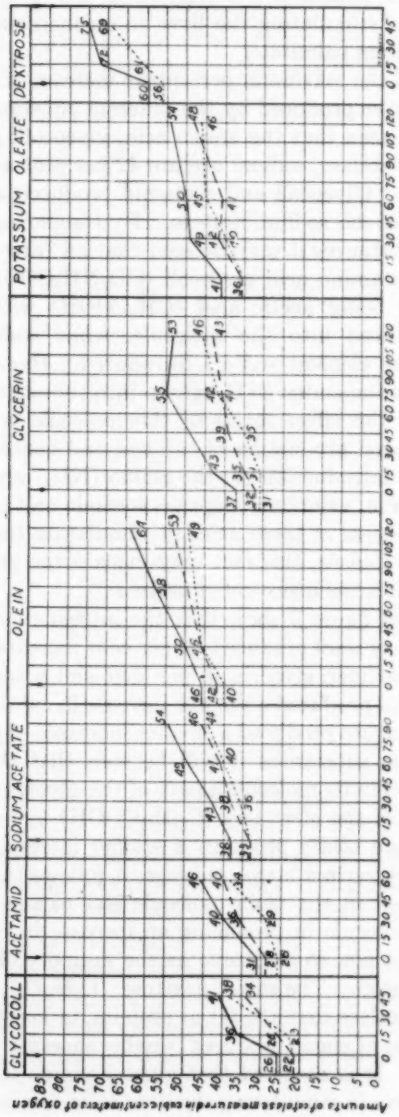
GLYCOCOLL
ACETAMID
SODIUM ACETATE
OLEIN
GLYCERIN
POTASSIUM OLEATE
DEXTROSE
Amounts of carbonic acid measured in cubic centimeters of oxygen
Time in minutes

Fig. 2

palmitate and for this reason it is assumed that the potassium oleate was more effective in stimulating the liver to an increased output of catalase than was sodium palmitate.

In figure 3 is given the maximum percentage increase in the catalase of the blood of the liver produced by the different substances given in figure 2. It may be seen that the amino acid, glycocoll, produced 57 per cent increase in catalase, sodium acetate 42 per cent and acetamid 48 per cent increase. Glycocoll and acetamid produced a greater increase in catalase than did sodium acetate. By comparing the formulae of these three substances it may be seen that all three are derived from acetic acid; the amino acid glycocoll (CH_2NH_2COOH) and acetamid (CH_3CONH_2) being acetic acid (CH_3COOH) with an amino (NH_2) group introduced into the molecule, while sodium acetate (CH_3COONa) has the element sodium introduced. Hence the effect of the introduction of the amino (NH_2) group into the acetic acid molecule was to increase the effectiveness of this molecule in stimulating the liver to an increased production of catalase and hence an increase in oxidation. If the introduction of the amino (NH_2) group into the organic acids, propionic, valerianic, caproic, succinic and glutaric, also increases the effectiveness of these acids in stimulating the liver to an increased output of catalase, this fact may explain the great increase in heat production after the ingestion of protein.

The curves given in figure 3 show that the introduction of olein, a fat, into the alimentary tract produced 40 per cent increase in the catalase of the blood of the liver, glycerin 48 per cent and potassium oleate 32 per cent. Glycerin produced a greater increase in catalase than did olein and potassium oleate produced a smaller increase. By comparing the formulae of these substances it will be seen that the fat, olein $(C_{17}H_{33}COO)_3C_3H_5$ has in its molecule a part of the glycerin $C_3H_5(OH)_3$ molecule and a part of the oleic acid ($C_{17}H_{33}COOH$) molecule. Since oleic acid or potassium oleate produces a smaller increase in catalase than the olein, and glycerin a larger increase, it follows that the effect of the glycerin radical in the olein molecule was to augment the effectiveness of this molecule in producing an increase in catalase in a manner similar to but not so extensive as the amino (NH_2) group in the amino acid, glycocoll. The sugar, dextrose, ($C_6H_{12}O_6$), produced a smaller increase in catalase than the fat or proteim, in keeping with the fact that the ingestion of sugar produces smaller increase in oxidation than fat or protein. It is assumed that glucose amine ($C_6H_{11}NH_2O_5$) would be more effective in stimulating the liver to an increased produc-

tion of catalase and hence in producing an increase in oxidation than glucose ($C_6H_{12}O_6$) owing to the presence of the amino (NH_2) group in the molecule. We were unable to procure any glucosamine and hence could not try out its effect on catalase production.

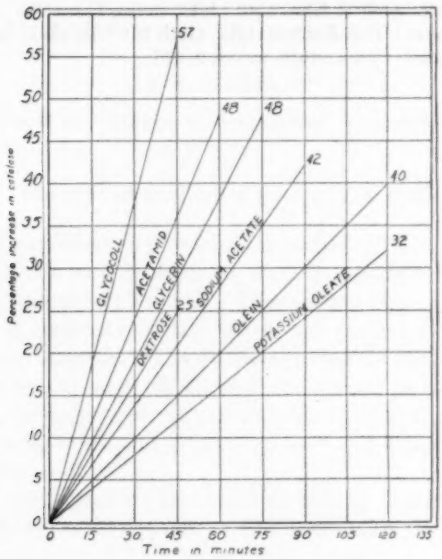

Fig. 3

SUMMARY

The introduction of small amounts of glutamic, aspartic and succinic acids, asparagin and acetamid produces no increase in catalase in keeping with Lusk's observation that small amounts of these substances do not increase oxidation. On the contrary, large amounts do produce an increase in catalase, in keeping with Grafe's observation that large amounts increase oxidation.

Evidence is presented to show that the amino (NH_2) group in the protein molecule renders protein, or meat, a more effective stimulant to catalase production and hence to heat production than fat and that the glycerine radical in the fat molecule renders fat more effective in this respect than sugar.

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ON THE STABILITY OF ESTERASE IN GROUND LIVER PRESERVED IN GLYCEROL

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In a series of experiments previously reported (1) the esterase content of the liver, spleen and kidneys in certain experimentally produced pathologic conditions was studied. These ground-up organs have now been standing in glycerol in a dark place, well corked, for from twenty-two to thirty-four months. The esterase content of the livers has again been tested, using the same dilutions of the extract as before. The results of these experiments are shown in table 1, from which it is evident that none of the specimens of ground-up liver had completely lost their esterolytic power.

The results may again be tabulated according to whether there has been *a*, an increase; *b*, no change; or *c*, a decrease in the ester-splitting capacity of the extracts. This is also shown in table 1. Of 22 extracts, 9 showed an increase, 7 showed a decrease and 6 showed little or no change in the amount of 0.1 N sodium hydroxide solution required to neutralize the acid liberated by the enzyme from ethyl acetate. It is evident, therefore, that at least for a period of thirty-four months the time element is not the most important factor in determining the diminution of esterase in ground liver preserved in glycerol.

The greatest increases noted were in extract IIIa from 3.45 cc. of 0.1 N sodium hydroxide in the first titration to 4.60 cc., and in control 3, from 3.45 cc. 0.1 N sodium hydroxide in the first titration to 4.80 cc. in the last titration after thirty-two and thirty-two and one-half months, respectively, in glycerol. The most marked decreases were shown by extracts Ia from 2.50 cc. 0.1 N sodium hydroxide to 1.15 cc. in the titration after thirty-two months in glycerol; and by sugar-fed 2 from 4.80 cc. 0.1 N sodium hydroxide to 2.60 cc. in the last titration after twenty-three and one-half months in glycerol.

TABLE 1

Showing the esterase content of ground-up liver preserved in glycerol for 21 days, and 22 to 34 months, respectively, expressed in cubic centimeters of 0.1 N sodium hydroxide required to neutralize the acid liberated from ethyl acetate

INCREASE			No change			DECREASE		
Animal	First titration	Last titration	Animal	First titration	Last titration	Animal	First titration	Last titration
In glycerol for from 30 to 34 months								
Control 1	3.35	3.80	Gl. 2	4.75	4.65	Chl. 2	3.40	2.90
Control 2	3.25	3.75	Pep. 2	4.90	4.90	Ib	3.55	3.20
Control 3	3.45	4.80	Ana. 1	3.90	4.00	Ia	2.50	1.15
Control 4	3.20	3.70	Ana. 2	4.10	4.00			
Gl. 1	4.60	5.00						
IIIa	3.45	4.60						
In glycerol for from 22 to 24 months								
IIb	4.15	4.40	IIc	4.30	4.35	S.F.1	4.60	4.10
Id	3.90	4.20	IId	3.75	3.65	IIa	4.40	3.95
Pep. 1	3.80	4.00				Ic	3.35	2.30
						S.F.2	4.80	2.60

The relation of the stability of the esterase in glycerol extracts of ground-up liver to the experimental procedure employed is shown in table 2.

DISCUSSION

It is evident from the above tables that ground-up liver preserved in glycerol may retain its esterolytic power unimpaired for a period of at least thirty-four months. The esterase content of the clear filtered glycerol extracts of liver may even be greater after many months' extraction than after the standard twenty-one days adopted in the previously reported experiments. That this was not due to any increased concentration resulting from evaporation was evident from the absence of any demonstrable loss of volume of the mass of tissue and glycerol.

The esterase in these livers is an intracellular enzyme. Duccheschi and Almagia (2) believe that cells yield their "lipase" (esterase) with some difficulty. It would appear reasonable, therefore, to suppose that the liberation of the enzyme might be related in a quantitative way to

the autolysis of the tissues being extracted. No tests were made to determine chemically the amount of autolysis that had taken place in these ground-up livers. In no instance was there complete liquefaction

TABLE 2

Showing the relation of the stability of esterase in glycerol extracts of liver to the pathologic condition experimentally produced

ANIMAL	FIRST TITRATION		LAST TITRATION	
	Days in glycerol	0.1 NaOH	Months in glycerol	0.1 NaOH
		cc.		cc.
Control 1	20	3.35	34½	3.80
Control 2	21	3.25	34	3.75
Control 3	21	3.45	23½	4.80
Control 4	21	3.20	34	3.70
Phosphorus poisoning Ia	21	2.50	32	1.15
Phosphorus poisoning Ib	22	3.55	31	3.20
Phosphorus poisoning Ic	21	3.35	22½	2.30
Phosphorus poisoning Id	20	3.90	22½	4.20
Phosphorus and sugar IIa	20	4.40	23½	3.95
Phosphorus and sugar IIb	20	4.15	23½	4.40
Phosphorus and sugar IIc	20	4.30	23½	4.35
Phosphorus and sugar IId	20	3.75	22½	3.65
Sugar-fed 1	23	4.60	23½	4.10
Sugar-fed 2	20	4.80	23½	2.60
Sugar-fed Gl. 1	21	4.60	32	5.00
Sugar-fed Gl. 2	21	4.75	32	4.65
Phosphorus and sugar recovered IIIa	21	3.45	23½	4.60
Chloroform 2	21	3.40	30½	2.90
Peptone poisoning 1	25	3.80	24	4.00
Peptone poisoning 2	21	4.90	30	4.90
Peptone poisoning 3	22		34	3.95
Anaphylactic shock 1	22	3.90	31	4.00
Anaphylactic shock 2	22	4.10	31	4.00

of the mass although it did appear to be softer and more finely divided after the prolonged extraction than after twenty-one days. There is evidence in numerous experiments recorded in the literature which jus-

tifies the belief that autolysis proceeded at different rates in the several groups of livers used in these experiments. Jacoby (3), Wells (4) and others have shown that phosphorus- and chloroform-poisoned livers autolyze much more rapidly than do normal livers. Evidence will be presented elsewhere (5) which appears to show that the rate of autolysis of liver is inversely proportional to the amount of glycogen present in the organ. A study of table 2 does reveal a relationship between the probable rate of autolysis in a given specimen and the increase or decrease of the esterolytic power of the extract. Thus, of four animals poisoned with phosphorus and whose livers might be expected to autolyze more completely, three showed a definite decrease in esterase content of the extracts; while of four dogs which were fed considerable quantities of sugar, whose livers contained large amounts of glycogen and would therefore autolyze more slowly, only one showed a decrease in esterolytic activity. Of four animals poisoned with phosphorus and fed with sugar, only one showed a decrease in esterase after prolonged extraction.

However, it does not necessarily follow that glycogen has a preservative action upon the esterase in prolonged extraction of ground-up liver in glycerol, for the conduct of the extracts of livers of control animals did not differ materially from that of the livers of sugar-fed dogs. But there is indication that the tendency to lose esterase is somewhat greater in the glycerol extracts of livers of sugar-fed dogs than in those of the controls.

In the previous paper (1) experiments were cited showing the inhibitory effect of bile upon the action of esterase. It seems probable, therefore, that the greater loss of esterolytic power in the livers of phosphorus-poisoned dogs may be related to the amount of bile present in the extracts which were definitely more deeply bile-stained than any of the others. This is indicated also by the fact that the greatest loss of esterase was in the liver of dog Ia which, in the earlier experiments, showed a lower esterase content than that of any other phosphorus-poisoned animal. This was explained in the previous paper as the probable result of the presence of the large amount of bile in the extract.

The extract of the liver of one of the sugar-fed dogs (sugar-fed 2) also showed a very marked loss of esterolytic power after twenty-three and one-half months in glycerol. No satisfactory explanation is offered for this apparent anomaly. If the results obtained from the extract of the liver of this animal are omitted, it is seen from table 2 that the average ester-splitting power of the livers of sugar-fed dogs is

still greater than that of the livers of the control animals, although the difference is not so great as in the case of the twenty-one-day extracts used in the experiments previously reported.

CONCLUSIONS

1. Ground dog's liver preserved in glycerol may retain its ester-splitting power unimpaired for a period of at least thirty-four months.
2. In some instances the clear filtered extract of the liver of a given animal shows a greater esterase content after prolonged standing in glycerol than after extraction for a period of twenty-one days. This increase is independent of the probable rate of autolysis of the specimen.
3. Diminution in the esterolytic power of the clear extracts is not related to the time during which the tissues have stood in glycerol, but to some other factor, possibly to the amount of bile present.
4. The feeding of sugar and the storage of the glycogen of the liver increases the ester-splitting activity of that organ.

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THE MECHANISM OF ETHER HYPERGLYCEMIA

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Received for publication January 3, 1919

In the course of studies on the relation of the hypophysis to hyperglycemia conducted by one of us (1), the influence of the ether anesthesia was the most difficult question to evaluate in the interpretation of the results. In the light of the existing literature, the influence of the anesthetic seemed of sufficient importance to warrant further investigation. Most of the data of the present paper was collected at that time but remained unpublished because of pressure of other work.

The ether glycosuria previously noted by Hawk (2) was shown to be associated with a hyperglycemia by Underhill (3) and Seelig (4). Seelig concluded that the glycosuria depends on quantity of ether used and duration of time through which it is administered, that it occurred regularly in animals fed on meat and in animals on carbohydrate diet only if anesthetic was administered within ten hours of last meal. Intravenous administration of oxygen prevented the appearance of glycosuria.

King (5) and associates reviewed the literature rather completely and made the first attempt to analyze the mechanism involved. They confirm the findings of Seelig that the depth and length of anesthesia are important modifying factors. In a later investigation King, Moyle and Haupt (6) induced anesthesia by the intravenous administration of ether in salt solution, and concluded that the site of action of ether is on the liver cells. A more detailed consideration of their findings will be presented later.

The more recent colorimetric methods of estimating blood sugar offer such advantages over the Oppler-Bertrand (7) method employed in these investigations that a repetition of a few of their experiments seemed to us advisable.

[1] The greater part of the experimental work of this paper was collected while both authors were members of Department of Pharmacology, Northwestern University Medical School.

Stewart and Rogoff (8) were able to produce hyperglycemia in animals by means of ether, asphyxia and so-called emotional states without the liberation of adrenalin in quantities sufficient to detect.

The mechanism of the hyperglycemia associated with the stimulation of the splanchnic nerve has been quite completely investigated by Macleod (9) and his coworkers. It furnishes an excellent basis for attacking the problem in hand. Stimulation of the splanchnic nerve gives a hyperglycemia. Stimulation of the hepatic nerves in the hepatic pedicle also gives a hyperglycemia if the adrenals are intact. If they have been removed from the circulation by ligatures the rise does not occur. The stimulation does not affect the quantity of glycogenase present. There is no absolute evidence to show whether these fibers are to be regarded as secretory or accomplish their effect through vasomotor changes. A small amount of adrenalin in the circulation appears to be a necessary factor in their activity.

Methods. The established effect of even slight degrees of asphyxia on the level of glycemia made it necessary at the outset to adopt all possible precautions against its occurrence. Unfortunately in a normal animal when an initial high level is once established it is not a transitory phenomenon but continues throughout the experiment.

The methods available for reducing a dog to the state of surgical anesthesia certainly suggest that the element of asphyxia must be a not inconsiderable factor at the outset. However, with the use of a towel folded so as to admit air rather freely this element can be reduced to a minimum. Once the animal was asleep the ether was administered by continuous insufflation, the tube being inserted well into the trachea. Such a method is of distinct value when procedures are contemplated that interfere in any way with respiration.

For this reason it was selected in our studies on the hypophysis and has been continued into the present series. Macleod (10) used oxygen insufflation in his earlier studies but he has come to discard entirely this principle in his later investigations. He feels that the possible alterations in the reaction of blood may introduce a larger source of error than the one corrected. In other words we may have an over-correction which is more confusing than a slight under-correction.

Samples of blood were drawn from the external jugular vein into oxalate crystals and the sugar was estimated according to the Benedict colorimetric method as modified by Meyers and Bailey (11). The time of the anesthesia was usually three hours with collection of samples just before ether, just after the establishment of insufflation and at one, two and three hour intervals.

The adrenalin was estimated according to method proposed by Folin, Cannon and Denis (12). The animals were fed a mixed diet containing a small amount of meat and an excess of carbohydrates. This diet has been shown by Ross and Hawk (13) and more recently by Ross

TABLE 1

Effect of ether administered by continuous insufflation on blood sugar of normal dogs

ANIMAL	BEFORE ETHER	AFTER ETHER			
		15 minutes	1 hour	2 hours	3 hours
1*	0.142	0.136			
	0.143	0.143	0.165		
2	0.102	0.184	0.191	0.213	
	0.101	0.183	0.200	0.209	
3	0.121	0.163	0.163		0.186
	0.120	0.167	0.163		0.182
4	0.114	0.206	0.196		
	0.116	0.180	0.192		0.192
5	0.105	0.116	0.178	0.220	0.220
	0.107	0.117	0.165	0.212	0.228
6	0.087	0.110	0.153	0.150	0.187
	0.093	0.113	0.156	0.157	0.165
7	0.086	0.117	0.195	0.215	0.203
	0.092	0.117	0.167	0.215	0.184
8	0.098	0.119	0.125	0.159	0.161
	0.099	0.125	0.124		0.146
9	0.101	0.146	0.175	0.216	0.237
	0.101	0.143	0.165	0.220	0.233
10	0.092	0.114	0.140	0.155	0.162
	0.092	0.093	0.136	0.157	0.160

* Dog fed same day, all others on day preceding.

and McGuigan (14) to be more conducive to a stable blood sugar level than one rich in protein.

Normal animals. Examination of table 1 shows that despite the precautions outlined above, the sugar level tends to a constantly higher

point reaching this at the end of the second hour. The third hour of etherization does not raise the level appreciably.

In four of the ten animals a concentration of 0.2 per cent or more was reached, a quantity which Macleod (15) has designated as a hyperglycemia.

Bilateral splanchnectomy. In animals with both splanchnic nerves (table 2) cut, the rise is confined to the early stages of anesthesia (fifteen minute period) and has worn off at end of two hours. Similar findings have been reported when the hypophysis was stimulated following complete section of splanchnics. We certainly can conclude that the mechanism of the persistent rise in glycemia is mediated through the splanchnic nerves. However, a transitory rise in the early stages

TABLE 2

Effect of ether administered by insufflation on doubly splanchnectomized dogs

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER			
			15 minutes	1 hour	2 hours	3 hours
11	7	0.102	0.139		0.101	0.109
		0.099	0.140		0.102	0.105
12	12	0.098	0.116		0.096	0.093
		0.099	0.121		0.097	0.089
13	90	0.102	0.145		0.117	0.115
		0.103	0.141		0.111	0.110
14	14	0.074	0.081		0.082	0.064
		0.075	0.082		0.080	0.064

may occur without its mediation. Nishi (16) has reported similar findings in studying diuretin hyperglycemia.

Unilateral splanchnectomy. Section of splanchnics on one side (table 3) does not abolish the rise in sugar. The level which is attained on the whole appears to be less than in the normals. In only one case (no. 22) was a concentration of 0.2 per cent reached. Animals 23, 24 and 27 are noticeably lower than normal. The findings in no. 21 may be disregarded since the animal was shown to have a localized peritoneal abscess despite which, however, he clinically was enjoying good health. All others were in good shape and had apparently completely recovered from the effects of the operation as shown by mental attitude, healing of abdominal incision and appetite.

TABLE 3

Effect of ether on blood sugar in animals with splanchnic nerves cut on one side

ANIMAL	DAYS SINCE OPERATION	SIDE OF OPERATION	BEFORE ETHER	AFTER ETHER		
				15 minutes	2 hours	3 hours
15	18	Left	0.091	0.127	0.116	0.135
			0.090	0.120	0.120	0.144
16	8	Left	0.084	0.101	0.122	0.155
			0.082	0.105	0.113	0.148
17	18	Left	0.097	0.134	0.190	0.181
			0.094	0.120	0.192	0.197
18	16	Right	0.083	0.098	0.155	0.160
			0.087	0.104	0.146	0.158
19	14	Right	0.074	0.145	0.176	0.152
			0.077	0.144	0.143	0.146
20	13	Right	0.092	0.093	0.122	0.171
			0.099	0.093	0.121	0.176
21*	11	Left	0.075	0.105	0.105	0.110
			0.080	0.105	0.099	0.100
22	11	Right	0.077	0.110	0.182	0.202
			0.079		0.172	0.184
23	9	Right	0.077	0.104	0.113	0.116
			0.082	0.109	0.109	0.106
24	12	Right	0.086	0.101	0.120	0.117
			0.086	0.105	0.111	0.110
25	18	Left	0.093	0.118	0.128	0.136
			0.088	0.115	0.119	0.131
26	12	Left	0.092	0.146	0.135	0.162
			0.095	0.129	0.133	0.147
27	11	Right	0.086	0.133	0.103	0.121
			0.091	0.127	0.102	0.118
28	11	Right	0.081	0.136	0.132	0.147
			0.080	0.132		0.136

* Abscess walled off by stomach, spleen and parietal peritoneum.

Hawk (2) has called attention to starvation as a predisposing factor to glycosuria. Had our results been in the other direction we should have been inclined to think that the animals had not returned to a normal metabolic level. We may consider that the mechanism has been modified by cutting off one of the final common pathways, a pro-

TABLE 4

Effect of ether on blood sugar in animals with denervated hepatic arteries

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER			
			15 minutes	1 hour	2 hours	3 hours
29	17	0.112	0.145	0.160		
		0.115	0.143	0.154		
30	14	0.136	0.273	0.273		
		0.137	0.273	0.240		
30*	20	0.107	0.172	0.255	0.317	
		0.101	0.168	0.255	0.322	
31	10	0.093	0.162	0.188	0.212	0.258
		0.090	0.165	0.188	0.216	0.242
31	29		0.116	0.111	0.153	0.156
			0.116	0.114	0.157	0.153
32	12	0.096	0.116	0.143	0.200	
		0.097	0.116	0.148	0.198	
33	12	0.097	0.136	0.190	0.190	
		0.095	0.134	0.178	0.184	
33	28		0.164	0.199	0.206	0.224
			0.164	0.206	0.194	0.218

* Fed on same day.

cedure which may possibly result in delimiting the afferent field but more probably exerts its influence on the effector side of the mechanism.

Certainly the splanchnic nerves appear to possess a quantitative influence and damage to any part of them would appear to have a mathematical influence on the glycemia. So far as our experiments go, we are unable to assign to either the right or the left side a more important valuation. This is in contrast to the findings of Nishi (16)

who states that section of left splanchnics in rabbits prevented diuretin hyperglycemia while division of the right side had no effect on its course.

Section of nerves of hepatic pedicle. In the first series of animals only the plexus of nerves attached to the hepatic artery was sectioned. The results of table 4 show that this has not modified the hyperglycemia. The suggestion even arises that the tendency to hyperglycemia is greater than normal.

TABLE 5

Effect of ether on blood sugar in animals with complete denervation of hepatic pedicle

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER		
			15 minutes	2 hours	3 hours
Approximately 40 cc. of blood withdrawn at each sample. Nutritional state normal					
34	17	0.089	0.110		0.175
		0.091	0.110		0.172
35	17	0.088	0.097		0.148
		0.083	0.097		0.140
36	17	0.078	0.095		0.135
		0.074	0.100		0.126
Nutritional state below par since last experiment					
34	20	0.119	0.191		0.252
		0.116	0.188		0.242
35	20	0.100	0.170		0.211
		0.095	0.182		0.211
36	20	0.091	0.154		0.144
		0.094	0.150		0.154
15 to 20 cc. of blood withdrawn at each sample. Nutritional state normal					
34	53	0.093	0.120	0.133	0.133
		0.095	0.114	0.137	0.132
35	53	0.073	0.094	0.102	0.127
		0.072	0.092	0.104	0.121
36	48	0.083	0.101		0.114
		0.089	0.094		0.121

TABLE 5—*Continued*

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER		
			15 minutes	2 hours	3 hours
Two 30 cc. samples withdrawn 3 hours previously, at 20 minute intervals; 10 cc. withdrawn for sugar estimation. Nutritional state normal					
34	59				0.139 0.132
35	59				0.142 0.145
36	59				0.078 0.081

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER 3 HOURS	
			Sample of 10 cc. withdrawn	60 cc. withdrawn and then a second 10 cc. for sugar estimation
35	73	0.103 0.093	0.127 0.110	0.166 0.168

Macleod (17) has shown that in denervating the liver he was able to get consistent results only after dividing all the structures of the hepatic pedicle except the portal vein and cauterizing this lightly. Our procedure involved the complete division of all tissues except the artery, vein and common duct and the cauterization of these. In the first three (table 5) the rise in blood sugar is evident and appears to be only slightly less than that occurring in normal animals.

A day was allowed to intervene and the experiment repeated. This group of experiments gave higher results, which we believe are to be attributed to conditions comparable to partial starvation as reported by Hawk (2). Further relatively larger samples (40 cc.) were withdrawn in both of these series. Repetition after feeding the animals for a number of days and withdrawing smaller samples (15 to 20 cc.) of blood gave a decidedly lower level in the next six experiments. Finally the effect of even a small hemorrhage is shown in the case of dog 35; after three hours of anesthesia in which only two samples of 10 cc. had been removed, the level of sugar was 0.127 and 0.110. Sixty cubic centimeters were withdrawn and the glycemia reached a point of 0.166 and 0.168.

King and associates (5) found a normal rise in glycemia after section of hepatic nerves. No mention is made of cauterizing the portal vein. They were also using a meat diet for their animals which, it will be recalled, is conducive to hyperglycemia. We may conclude that these animals show a marked glycemic response to hemorrhage even in small amounts but the response to ether is less than in unoperated animals.

Eck fistula. King and associates (5) found that a hyperglycemia occurred in dogs upon which an Eck fistula had been established. The level of this was slightly over 0.1 per cent for two hours of ether administration (four experiments).

Table 6 shows that in a period of three hours the rise may be quite as high if not higher than normal, though the effect may not be so ap-

TABLE 6

Effect of ether on blood sugar in dogs with Eck fistula

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER	
			15 minutes	3 hours
37	30	0.094	0.095	0.190
		0.092	0.097	0.186
38	28	0.110	0.125	0.171
		0.111	0.116	0.178
38	41	0.108	0.116	0.241
		0.107	0.126	0.251
39	26	0.102	0.132	0.218
		0.095	0.131	0.215

parent in the first fifteen minutes. It is highly probable that the operation has not modified the course of events but on account of the smaller blood supply to the liver a longer time is required in which to transfer the excess sugar to the circulation.

Macleod has reported that the acute effect of Eck fistula operation is an acceleration of the glycogenolytic processes. McGuigan and Ross (18) studied same question reporting a hyperglycemia. However our experiments are not directed toward this end. We were endeavoring to see how a liver whose circulation was thus crippled would respond to ether stimulation, and our data show that a hyperglycemia of the same or even greater magnitude than normal may be attained.

Ligation of hepatic artery in Eck fistula animals. Since the animals were available and we desired to get some knowledge of the efficiency

of the collateral circulation, the hepatic artery was ligated and the blood sugar followed until death. McGuigan and Ross (18) have reviewed the literature of this subject and reported findings similar to those recorded below. Since their ligations of two vessels were performed within a short time of each other it seemed worth while to record present data obtained by succeeding ligations. The protocols are attached.

Effect of ligation of hepatic artery in dogs with Eck fistula

Dog. 37. February 3, 1918

Time since operation	49 days
Blood sugar estimations before anesthetic	0.104 0.091
After anesthetic just before ligation	0.191 0.200
Four hours after ligation	0.032 0.032
Five hours and ten minutes after ligation just before death	0.018 0.018

Dog 38. February 20, 1918

Time since operation	68 days
Estimations of blood sugar before anesthetic	0.093 0.085
After ether just before ligation of artery	0.128 0.120
One and one-half hour after ligation	0.128 0.130
Two and one-half hours after ligation	0.103 0.106
Five hours	0.093 0.097
Twenty-four hours	0.095 0.097
Thirty hours, just at time of death	0.043 0.039

Dog 39. February 20, 1918

Time since operation	66 days
Blood sugar estimations before anesthetic	0.088 0.088
After anesthetic, just before ligation of hepatic artery	0.124 0.126
One and one-half hour after ligation of artery	0.110 0.107
Four hours after ligation	0.091 0.092
Six and one-half hours after ligation	0.061 0.062
Animal died during night	

The first animal (dog 37) died rather quickly (five hours and ten minutes). The blood sugar promptly fell to a level of 0.018.

In dog 38 the hypoglycemia appeared during the last six hours of life; in dog 39 it was definitely present in a sample taken at six and one-half hours. The animal died sometime during the night. It is evident that the collateral circulation was insignificant and that the hypoglycemia as has been previously shown is a part of the terminal syndrome.

Reversed Eck fistula. In these animals the anastomosis between the two veins was performed as usual but the ligature was placed about the vena cava thus directing all the venous blood through the liver.

McGuigan and Ross (18) have reported that this procedure per se does not cause a rise in glycemia.

Our experiments (table 7) show that ether causes a rise comparable in all respects to that found in normal animals.

TABLE 7

Effect of ether on blood sugar in dogs with reversed Eck fistula

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER	
			15 minutes	3 hours
40	26	0.078	0.117	0.128
		0.079	0.113	0.135
41	27	0.132	0.185	0.207
		0.131	0.182	0.202
42	29	0.094	0.140	0.145
		0.092	0.139	0.142

Adrenalin content in unilaterally splanchnectomized animals. The most mooted question is the part played by the adrenalin in the hyperglycemia. Examination of table 8 shows that in four cases (17, 18, 26, 27) there was no significant difference in the load of the two glands. In four others (16, 19, 20, 23) the control gland had apparently a higher content, in the remaining six the content was less in the control than the experimental gland.

Elliott (19) has shown for cats that ether exhausts the content of adrenalin in a gland which has not been protected by section of its splanchnic nerves. Having in mind this investigation, we hoped to determine whether a relationship existed between the rise in sugar and the degree of exhaustion. A comparison of tables 3 and 8 shows no relationship whatever exists. It is possible that in the dog some of the

fibers of the right side may have crossed to the left adrenal. We believe however that our section would have included these. More probably the ether causes an increased production as well as increased partition and it is on this basis that the inconclusive results are to be explained.

They were consequently not extended to a larger series of cases. The partition of adrenalin from the intact glands under ether anesthesia has been abundantly established by Stewart (20) and his associates. Our results are to be taken as further confirmation that ether is modifying the activity of the gland. The question at issue concerns not the

TABLE 8

Adrenalin load in glands at end of 3 hours of ether insufflation*

ANIMAL	DAYS SINCE SPLANCHNECTOMY OPERATION	EXPERIMENTAL GLAND	CONTROL GLAND	CONTROL / EXPERIMENTAL: EXPRESSED IN PERCENTAGE
				per cent
15	18	0.99 right	0.65 left	65
16	8	0.35 right	0.54 left	154
17	18	0.74 right	0.78 left	105
18	16	1.06 left	1.08 right	101
19	14	0.14 left	0.33 right	235
20	13	0.69 left	0.82 right	118
21	11	0.60 right	0.39 left	65
22	11	1.20 left	1.06 right	88
23	9	0.67 left	0.77 right	115
24	12	1.08 left	0.89 right	82
25	18	0.88 right	0.86 left	97
26	12	1.34 right	1.46 left	109
27	11	0.53 left	0.45 right	84
28	11	0.84 left	0.53 right	63

* Results expressed in milligrams.

partition of the adrenalin but the use of the adrenalin after it enters the blood stream. Is it there because its secretion and the glycogenolytic processes happen to be controlled by two functionally different classes of fibers running in the same nerve which are being commonly stimulated? Is it there to complete the activity of the fibers governing the glycogenolysis?

Stewart and Rogoff (8) conclude that it is not a necessary factor since the ether rise can occur without its demonstrable presence. Of four of their experiments only two show a gain (0.087 to 0.151 and 0.142 to 0.176) that does not fall within the limit of experimental error. The

other two (0.200 to 0.233 and 0.092 to 0.098) are not significant. The time of their anesthetic was fifteen to twenty minutes.

It will be recalled that in our cases, those animals with bilateral splanchnectomy, a similar early (15-minute period) rise was noted. This however passed off at the end of two hours. Such a rise might be very readily explained on basis of asphyxia which unquestionably should be attributed in a great measure to an altered reaction of the blood. We do not feel that their experiments establish the case against the adrenals playing a part in the ether hyperglycemia unless they show that "the persistent long rise" can occur without their mediation. This experimental condition they have not investigated.

DISCUSSION

That the liver is the ultimate effector organ upon which the stimulus falls is certain from the studies of numerous observers who have investigated the nervous hyperglycemias. As to the intrinsic mechanism involved by which these nervous stimuli result in an increased glycogenolysis we have not concerned ourselves. King, Moyle and Haupt (6) in their investigation, using intravenous ether saline solutions, have concluded that the liver cells are the site of the ether action. The hyperglycemia did not occur until surgical anesthesia was produced. The question arises whether the two conditions are identical. It is not at all certain that the ether introduced through the respiratory tract arrives at the liver in the same concentration as it would were it introduced intravenously. It may be more toxic in this latter state. Indeed the absence of hyperglycemia in bilaterally splanchnectomized animals seems to us to constitute final proof that the site of action is not on the liver cells. For these reasons we believe that they have not answered the question in hand but an entirely different one raised by a new experimental procedure.

The extrinsic mechanism in which we are interested involves the splanchnic nerves with their terminations in the liver and adrenals and the discharge of small amounts of adrenalin. The established tonic effect of adrenalin on sympathetic terminations in other sites suggests strongly that it has a value here. The maintenance of a hyperglycemia (of a lower grade, it is true) with the adrenals intact but liver denervated, furnishes to our minds rather definite evidence that the adrenalin is an essential factor. One can object that in such animals the whole abdominal viscera are exposed to vascular alterations. However very

extensive changes in circulation through the liver have not been shown to be accompanied by significant variations in glycemia. We must in our thinking push the site of action of these influences beyond a consideration of the quantity of blood delivered passively to the liver, to a consideration of some more active participation on the part of the liver capillaries and cells. It is in controlling this more active participation that we believe the hepatic splanchnic nerves and the adrenalin play their respective parts. Indeed we believe the mechanism to be identical for ether and electrical stimulation of splanchnic nerve, as reported by Macleod (9). They are only two different exciting agents which are capable of throwing into activity the same effectors.

CONCLUSIONS

1. A persistent hyperglycemia occurs in normal dogs under continuous ether insufflation. This reaches a maximum at end of two hours with little change in the third hour.

2. A transient hyperglycemia passing off at the end of two hours occurs in animals with splanchnic nerves sectioned on both sides.

3. A persistent hyperglycemia of a lower grade occurs in animals unilaterally splanchnectomized. There is no difference in the value of the nerves of the right and left sides.

4. Denervation of the hepatic artery gives a glycemic rise equal to that of normal dogs.

5. Section of all the nerves in the hepatic pedicle with cauterization of the coats of hepatic artery, portal vein and common duct prevents the sugar rise from reaching the high point found in unoperated animals but does not abolish it.

6. Eck fistula does not prevent but only retards the appearance of hyperglycemia in the systemic veins.

7. Ligation of hepatic artery in Eck fistula animals causes a terminal hypoglycemia.

8. Reversed Eck fistula does not influence course of ether hyperglycemia.

9. The adrenalin content in dogs whose splanchnic nerves have been previously sectioned on one side is unequal in the two glands.

10. The partition of adrenalin under ether is considered of significance and not an incident of splanchnic stimulation.

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STIMULATION BY THE OUTWARD DIFFUSION OF AN ELECTROLYTE FROM THE IRRITABLE TISSUE

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The peculiar physiological behavior of barium among the alkaline earth metals has long been known. Its greater toxicity, compared with the other alkaline earths, has been investigated by many, especially by Overton (1) who showed that a sartorius muscle in 0.7 per cent NaCl plus 1: 10,000 $BaCl_2$ loses all its irritability in four and one-half hours. Correspondingly, the antitoxic action of barium toward other salts is much less than that of calcium or strontium.

The power of barium to cause rhythmical contraction of striated muscles was established in 1886 by Ringer (2); this was subsequently investigated more fully by Loeb (3). In this respect especially does barium differ radically from calcium and strontium, for it is well known that the twitches in a skeletal muscle caused, for example, by sodium salts can readily be prevented by the addition of calcium or strontium salts to the stimulating solution.

The rhythmical contractions produced by barium salts can also be prevented by the addition of salts of other metals. Ringer showed this to be true for the chlorides of potassium and calcium. I found that 7 cc. m/8 $BaCl_2$ diluted with 3 cc. m/8 KCl is unable to call forth any rhythmical activity (4) although $\frac{1}{8}$ cc. and even $\frac{1}{16}$ cc. m/8 $BaCl_2$ in 10 cc. m/8 NaCl speedily throws the muscle into violent twitches. The present experiments showed that this antagonism toward barium is also shared by the chlorides of strontium and ammonium, to a lesser extent by lithium chloride and by hydrochloric acid. Sodium chloride and more especially sodium hydroxide favor the stimulating action of barium. A muscle previously treated with 0.7 per cent NaCl for thirty or forty minutes responds more energetically to a dilute solution of barium chloride and with a shorter latent period than a muscle not thus treated. This favorable action of sodium chloride is well shown by bathing the muscle in a 6 per cent cane sugar solution previous to its

immersion in the barium salt. One of the gastrocnemius muscles which had remained for twenty minutes in 6 per cent cane sugar solution was placed in 1 cc. m/8 $BaCl_2$ plus 9 cc. 6 per cent cane sugar; after a latent period of eighteen minutes a few twitches occurred. The other gastrocnemius muscle, after having remained in m/8 NaCl for eighteen minutes, on immersing in a solution of 1 cc. m/8 $BaCl_2$ plus 9 cc. m/8 NaCl began to contract rhythmically within two minutes and continued to twitch violently for a long time. This action of the cane sugar is due, as Overton states (5), to the removal of the sodium salts from the muscle for on bathing the sugar-treated muscle for a sufficient length of time with 0.7 per cent NaCl the response to barium is normal. This, however, does not give us reason to state, as Overton (1) seems to do, that barium is not a stimulus for the striated muscle. While it is no doubt true that barium has no stimulating effect on a muscle except in the presence of sodium salts, it is also true that a muscle which has remained for some time in a solution of cane sugar loses its irritability to the faradic current. Such a muscle is no longer a normal muscle. Hence we hold that barium salts have a powerful stimulating effect on striated muscles.

Whether the stimulating action of the barium and the antagonistic action of potassium, calcium and other salts are due to effects upon the muscle, nerve or myoneural junction I did not determine, being unable to procure curare. Edmunds and Roth (6) state that curare does not influence the course of the contraction caused by barium chloride in muscles of fowls and that denervation has no effect either; they therefore conclude that barium acts on the muscle cells directly.

The antagonistic action of a compound is demonstrated not only by the fact that the minimal concentration of the barium chloride necessary for stimulation is increased when the muscle has been previously treated with the antagonistic salt or when this salt is used simultaneously with the barium, but also by the abolishing of the contractions by the counteracting solutions when once the twitches have been established by the barium. This can, in case of the barium twitches, be readily demonstrated by placing the actively contracting muscle in m/8 calcium chloride solution. Wishing to prove this also for potassium chloride, a muscle showing a fair degree of activity in a m/160 $BaCl_2$ solution was transferred to a m/8 KCl. To my surprise, instead of an almost immediate inhibition of the twitches as I had been led to expect from previous experiments with this salt, the muscle was thrown into far greater twitches than it exhibited previous to its immersion in the po-

tassium chloride solution. In figure 1 the contractions from *a* to *b* were produced by the muscle in 1 cc. m/8 $BaCl_2$ plus 9 cc. m/8 NaCl; at *b* this solution was replaced by 2 cc. m/8 KCl plus 8 cc. m/8 NaCl. In the other experiment a pure m/8 KCl solution was used with practically the same initial result. Having made certain that this was no accidental affair, I made experiments with other solutions and found that m/8 sodium chloride had a similar but much more prolonged effect. In fact, when a muscle is left long enough in a barium chloride solution for the twitches to cease, the subsequent bathing of the muscle with m/8 sodium chloride causes energetic contractions. In figure 2 the twitches in the dilute barium chloride had practically ceased at *c;* on the application of m/8 sodium chloride at *d* the muscle is thrown into violent twitches.

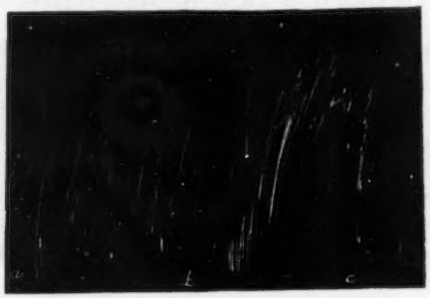

Fig. 1. At *a* the muscle is placed in 1 cc. m/8 $BaCl_2$ + 9 cc. m/8 NaCl; at *b* this is replaced with 2 cc. KCl + 8 cc. NaCl. At *c*, same as at *a*.

In seeking an explanation for the reëstablishing of these twitches when the stimulating agent is replaced with an indifferent or even antagonistic solution, many suggested themselves. At first I was inclined to regard this as an example of the so-called "contact irritability" (7), (8) by which a muscle treated with certain salts is thrown into great activity when the exciting fluid is replaced with almost any other fluid. However, while a muscle showing contact irritability goes into strong rhythmical contractions when it is brought from the sensitizing solution (e.g., sodium citrate) into contact with air, this is not necessarily true for a muscle treated with barium chloride.

Neither can the cause of these contractions in the sodium chloride solution be sought in the mechanical stimulation brought about by the removing of the barium chloride and its replacement with a sodium

chloride solution, for the mere removal and return of the barium solution in which the muscle has almost ceased to contract does not reëstablish the twitches. Moreover, experiments to be related presently speak against the view that mechanical stimulation has anything to do with this phenomenon.

Seeing that we are replacing a bivalent kation, Ba, with a univalent kation, Na or K, it might be surmised that this may be the cause of the stimulation. However, a few experiments made with calcium chloride render this very unlikely. In a number of these experiments the change from barium to calcium chloride or magnesium chloride was followed by a slight increase in the twitches, or at least a far better continuation of the twitches than occurs when the muscle is merely taken from the barium chloride and left in the air. Of course, this increase in the contractions is limited as might be expected from the marked antagonism existing between barium and calcium in this matter. And again, in favorable instances the replacement of the barium chloride with 6 per cent cane sugar solution has an effect similar to that shown in figure 2, where the sodium chloride is substituted for the barium. But in the case of cane sugar, as with the calcium chloride, the resulting twitches are of shorter duration, no doubt due to the lack of sufficient sodium salts. As the substitution of either the univalent sodium or the bivalent calcium or even a non-electrolyte for the barium brings about the same qualitative results, these results cannot be attributed to the electric charges of the substances concerned.

Neither is it likely that the cessation of the twitches in the barium chloride is due to the establishment of a physiological balance between the sodium and barium in the muscle and that therefore the influx of sodium, upsetting this balance, causes anew the stimulation of the muscle. When a muscle which has ceased to contract in a solution composed of 10 cc. m/8 NaCl plus $\frac{1}{8}$ cc. m/8 $BaCl_2$ is bathed with 10 cc. m/4 NaCl plus $\frac{1}{8}$ cc. m/4 $BaCl_2$ solution the twitches begin once more, although the proportion of sodium to barium has not been altered. Let us suppose that the original stimulating solution is composed of 9.5 cc. m/8 NaCl plus 0.5 cc. $BaCl_2$; in this the twitches will finally cease. Now it is immaterial whether we substitute 10 cc. m/8 NaCl, 5 cc. m/8 NaCl plus 5 cc. 6 per cent cane sugar, 10 cc. NaCl plus $\frac{1}{16}$ cc. $BaCl_2$ or 8 cc. NaCl plus 2 cc. $BaCl_2$; in every instance the muscle shows increased activity. While it is conceivable that a physiological balance of sodium and barium might result in an inhibition of twitches, it is difficult to see how the upsetting of this equilibrium either by an increase or a de-

crease in the barium or in the sodium should in all cases act as a stimulation. To do so would be to suppose that barium in a certain concentration has an inhibiting influence over the stimulating action of sodium chloride and that the sodium in this same concentration prevented the barium from stimulating. For such mutually inhibiting qualities we have found no evidence.

That the barium chloride has made the muscle more irritable toward the sodium chloride is hardly likely to be the cause of the stimulation when the barium chloride is replaced with sodium chloride for, as I have already stated, the stimulation takes place whether we introduce a sodium chloride of greater or of lesser concentration than that with which the stimulating barium chloride solution was diluted. And the fact that in favorable cases a sugar or a potassium chloride solution is able to evoke the contractions after the muscle has been treated with a

Fig. 2. From *e* to *a* the muscle is placed in 0.5 cc. m/8 $BaCl_2$ + 9.5 cc. m/8 NaCl. At *a* and *c* m/8 NaCl is applied; at *b* and *d* the m/8 NaCl is replaced with 0.5 cc. $BaCl_2$ + 9.5 cc. NaCl.

barium salt speaks against the view that increased irritability toward sodium is the cause of these renewed twitches.

It is therefore not the upsetting of a physiological balance of barium and sodium nor the passage of sodium into or out of the muscle that can be regarded as the stimulating agency. The results of the experiments related seem to be explicable only on the theory that the actual passage of the barium salt into or out of the muscle and not its mere presence in the muscle stimulates. To prove this four or five experiments were made.

1. When a muscle has become quiet in a dilute barium chloride solution, the immersing of the muscle in a sodium chloride solution reinstates the twitches. If now, while the twitches thus caused are at their highest, the sodium chloride solution is removed and the barium chloride is replaced, the twitches cease very speedily. In figure 2 the twitches

following *a* and *c* are due to the substitution of 10 cc. m/8 NaCl for 0.5 cc. m/8 $BaCl_2$ plus 9.5 m/8 NaCl. When the twitches were well marked, at *b* and *d*, the sodium chloride was removed and 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl substituted. It will be seen that the twitches cease almost immediately.

2. The two gastrocnemius muscles were prepared and placed in 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl; in figures 3 and 4 the initial twitches are

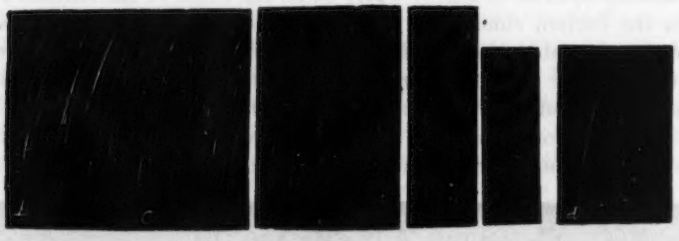

Fig. 3. From *b* to *c* the muscle is placed in 0.5 cc. m/8 $BaCl_2$ + 9 5 cc. m/8 NaCl. At *c* this is replaced with 10 cc. m/8 NaCl in which the muscle remains till *d* when the original solution is replaced.

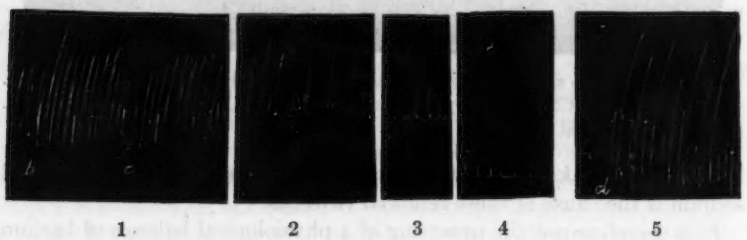

Fig. 4. From *b* to *c* the muscle is placed in 0.5 cc. m/8 $BaCl_2$ + 9.5 cc. m/8 NaCl. At *c* this is replaced with NaCl which is continually renewed. At *d* the NaCl is replaced with 0.5 cc. $BaCl_2$ + 9.5 cc. NaCl.

those caused by this solution. At *c*, in both figures, the barium chloride is removed (after having acted for thirty minutes) and replaced with sodium chloride. In figure 3 this sodium chloride is not renewed but in figure 4 a constant renewal takes place. The parts of the two curves numbered from *1* to *5* correspond in time. It will be seen that although the muscle in figure 3 was originally more active than its mate, fourteen minutes after the application of the sodium chloride

(in section 2) the twitches cease while they continue for ten minutes longer in figure 4. When the sodium chloride solution is not renewed an equilibrium between the barium salts in the muscle and in the surrounding fluid is soon established; the outward diffusion of the barium salt then ceases and the muscle is no longer stimulated. On the other hand, when the muscle is constantly supplied with fresh sodium chloride solution the diffusion of the barium salt out of the muscle continues for a longer length of time and hence the stimulation is also of greater duration, this ceasing when all the salt has been removed.

3. This theory of stimulation by the outward diffusion of the barium is further supported by the continuation of the above experiments illustrated in figures 3 and 4. When both muscles had become quiescent in the sodium chloride solution, at *d* figures 3 and 4, this solution is removed and the original solution of 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl substituted. In figure 3, in which previous to *d* the sodium chloride solution had not been renewed, there is little or no activity. On the other hand, the muscle which had been bathed continually with fresh sodium chloride solution almost immediately goes into strong contractions when treated with the original barium solution at *d*, figure 4. In figure 3, the presence of a considerable amount of barium chloride in the muscle prevents the rapid and extensive passage of this salt into the muscle and consequently this muscle is not stimulated to any great extent. But when by the proper washing with a large volume of sodium chloride solution the barium salt is removed from the muscle (fig. 4), the subsequent application of barium chloride is followed by a quick entrance of this salt into the muscle and, as a result, stimulation occurs.

4. In another experiment the two muscles are placed in the proper concentration of barium chloride. After a certain time this is replaced with a sodium chloride solution. In one muscle, say A, this sodium chloride solution is not renewed and the twitches, as related above, speedily come to a standstill. The other muscle, B, is continually washed with fresh sodium chloride solution and its twitches continue for a much longer length of time than those of A. If now, while muscle B is still actively twitching in the sodium chloride solution, this solution is removed and replaced with the sodium chloride solution in which muscle A has ceased its activity, muscle B also very soon becomes quiet. Evidently a sufficient amount of barium salt had diffused from muscle A into the sodium chloride solution to stop the diffusion of the barium salt from muscle B when it is placed in this solution; as the outward diffusion of barium ceased, the stimulation also ceased.

5. It is of interest to compare the results of the application of dilute barium chloride at *b* and *d* in figure 2 with those obtained at *d* in figure 4. In figure 2 the muscle is energetically twitching in the sodium chloride which has been applied for only about a minute; the barium salt is still actively diffusing from the muscle and has by no means reached the end; the application of the barium chloride stops this outward diffusion, but because of the relatively large amount of barium in the muscle, no very active entrance of barium into the muscle is possible and hence there is no stimulation. But in figure 4 the sodium chloride solution having been frequently renewed has removed all the barium from the muscle and hence immersing the muscle in a dilute barium chloride solution at *d*, causes the passage of barium into the muscle; this results in stimulation.

6. That the twitches ensuing upon the replacement of barium chloride with sodium chloride are in reality due to stimulation by barium chloride is rendered highly likely by the fact that when the solution following the barium is composed of potassium chloride or calcium chloride (diluted with sodium chloride), there is at first an increase in the muscle activity, but this activity ceases in most instances very abruptly because of the great antagonism existing between the barium and the potassium or calcium salts.

On looking through the literature for other instances where not the mere presence of the stimulating agent in the tissue but its actual passage into or out of the tissue stimulates, I was able to find only two or three references to this matter. In 1907 Straube (9) found that muscarin which had already penetrated the heart was without physiological action. This investigator placed the heart in a solution of muscarin; after a certain period of inhibition spontaneous recovery took place. At the time of the resumption of the heart beat, the blood in the heart and extracts made from the heart contained sufficient muscarin to inhibit a freshly excised heart. Straube however does not speak of the removal of the muscarin from the heart.

Neukirch (10) observed that the tonus induced in the musculature of the small intestines by means of pilocarpin was increased when the pilocarpin was removed by bathing the tissue with water. This stimulating action Neukirch attributes to the outward diffusion of the pilocarpin from the tissue, but how this causes stimulation he does not attempt to explain. Somewhat similar effects were noted by Meyer (11) in the action of eucain and suprarenin on the contraction of the musculature of blood vessels. In all of these investigations the stimulating agencies

employed were complex organic compounds and the methods of experimentation more or less complicated.

The question of why the static presence of an introduced electrolyte in a muscle does not stimulate while its actively passing into or out of the muscle is accompanied by increased activity is naturally but one phase of the general question of what constitutes stimulation. According to the "membrane theory" of Overton, Hoeber, Lillie and others, an increase in the permeability of the plasma membrane is one of the first links in the chain of events which finally finds expression in the peculiar activity of the tissue. When this increase in permeability is abnormally great the stimulation gives place to toxic action (12). It seems difficult to harmonize this theory with the above described results. Let us suppose a muscle-nerve preparation is placed in 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl. After a latent period varying from one-half to five minutes, vigorous contractions take place, which cease after a certain length of time. The stimulation producing this activity is attributed to an increase in the permeability of the plasma membrane. The subsequent cessation of activity might be explained by supposing that a lower concentration of the barium salt on the surface of the membrane increases the permeability but that a higher concentration decreases this property. Aside from the fact that at present there is no evidence for this, how can we explain that this quiescent muscle, if placed in a more concentrated barium chloride solution, renews its twitches? And if, according to Lillie, the parallelism existing between the stimulating power and the toxicity of chemical compounds is due to qualitatively similar effects on the permeability of the protoplasmic surface film, the matter is still more complicated. For if a muscle ceases its activity in a dilute barium chloride solution because the concentration of the barium in the muscle is so great as to reverse its usual action on the plasma membrane and thus to decrease its permeability, how then, according to the membrane theory, can the continued presence of the barium in this concentration exert any toxic influence? If it is argued that the continued presence of the barium gradually increases the permeability and thereby destroys the integrity of the cell (for which reason the stimulation and increased activity cease and the cell gradually dies), the only way in which the removal of the barium can renew the stimulation is by restoring the permeability to its normal condition, i.e., by decreasing the permeability. But according to the membrane theory (13), a decrease in permeability causes not stimulation but inhibition.

SUMMARY

1. Barium chloride does not stimulate by its mere presence in the irritable structure but by its active diffusion into or out of the tissue. It is therefore possible to cause cessation of the rhythmical contractions by immersing the muscle in a barium solution which originally caused rhythmical activity.

2. The stimulating action of barium chloride is counteracted by the chloride of potassium, ammonium, calcium and magnesium. Sodium chloride increases the effect of barium salts.

3. Bathing a muscle with 6 per cent cane sugar solution speedily reduces its irritability toward barium salts, due to the removal of sodium salts from the muscle.

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OBSERVATIONS ON THE CATALYTIC POWER OF BLOOD AND SOLID TISSUE

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At frequent intervals during the past year there have appeared in the literature publications regarding the substances in the blood and body tissues which have the power of releasing oxygen from hydrogen peroxide. The results published have shown a remarkable uniformity, and from the interpretation placed upon them the reader is led to infer that the activity of these enzymes is responsible for many of the physiological processes of the body.

According to Burge, the variations in the activity of these enzymes explain, among others, these phenomena: *a*, The physiological differences between used and unused muscles (1); *b*, Differences between the ratios of heat production (oxidation) in large and small animals (2); *c*, The loss of weight during starvation (3); *d*, Loss of weight during thyroid feeding (4); *e*, The mechanism of phosphorus poisoning (5); *f*, Certain body changes due to emotions (6); *g*, Mechanism of action of anesthetics (7), (8), (10); *h*, Shock (9); *i*, The specific dynamic action of food in the sense of Rubner (11), (12), (13); *j*, The physiological basis for the therapeutic use of alcohol in diabetes (14); *k*, A therapeutic basis for the use of saccharin as a food.

What seemed *a priori* like an error in reasoning in one of the earlier papers (5) of this series led the writer to repeat a few of the experiments, the recent editorial in one of the widely read medical publications (16) led to the completion of this article and its publication.

The substances releasing oxygen from hydrogen peroxide we will call *catalase*, with the understanding that the term is used in the sense suggested by Kastle (17), and the use of the term in no way commits us to any view regarding the nature of the substances concerned. The usual and probably the correct view is that the catalase is an enzyme widely distributed in nature.

The views regarding the function of catalase in the animal economy are as follows: Loew (18), the pioneer in the field—believes the function of catalase to be the destruction of any peroxides formed during the life of the cell, which by their activity would prove harmful to the organism. This same view was advocated by Hertzlika (19) and Shaffer (20). Batelli and Stern (21) observing that catalase inhibits certain oxidations by ferrous sulphate, believe that the function of catalase is the protection of the body by the prevention of excessive oxidation within the cell. Jolles and Oppenheim (22) see in the catalase the substance which releases oxygen from hemoglobin for use by the cell. The same view was advocated later by Ewald (23). Burge (1) claims that measuring the catalytic power of the tissue of an animal measures the rate of metabolism of the tissue. This view was based upon the observation that, in his determinations, the catalytic power of an active muscle like the heart is greater than the catalytic power of less active muscles like those of the leg; while the latter show greater catalytic power than the still less active muscles of the head. Further (2) starting with the well-known fact that the oxidative power of small animals is greater than the oxidative power of larger ones, he studied the heart muscle of birds of various sizes: goose, pigeon and sparrow, and of mammals of various sizes: dog, rabbit and rat, and found that the content of the heart muscle in catalase bears an inverse relationship to the size of the animal. From these observations he concludes that a measure of the catalytic power is a measure of the metabolic activity of a tissue.

Inability to reproduce the results obtained by Burge in some of his early papers led us to a study of various types of apparatus until we finally adopted one which gives results with errors varying between 0 and 9 per cent for blood. Usually the error is less than 3 per cent. It is impossible from reading Burge's papers most carefully to arrive at any conclusion regarding the number of determinations made on each sample and therefore we have no way of estimating a fact not stated in the text; viz., the percentage of error in the method he employed.

1. Method. Our apparatus has undergone various developments but, as completed, it consists of six gas burettes each of 250 cc. capacity, provided with a side arm 10 cm. above the open end and a glass stop cock at the upper end. These burettes are fastened open end down on a ring stand. The open end is closed with a rubber stopper through which passes the upright limb of a glass T-piece. The ends of the cross bar of the T-tube are fastened to the correspond-

ing ends from the next burette. In this way the six burettes are all connected with one another. Then the open ends of the end T-pieces are connected with each other by rubber tubing, and the tubing is connected with a 4 liter Mariotte bottle full of distilled water saturated with oxygen. By raising this Mariotte bottle all the burettes can be filled by closing the side arms and opening the glass stop cocks at the top. The pressure of gas inside and outside the burettes can also be equalized after the evolution of oxygen has ceased, by raising and lowering the bottle to the proper level. During the release of oxygen the bottle is placed on the table beside the burettes. To the side arm of each burette is attached a piece of rubber tubing about 18 inches long. This is closed by a pinch clamp except during the release of oxygen. The free end of this tubing is slipped over the side arm of a Bunsen gas washing bottle of 125 cc. capacity. When ready for a test 50 cc. of hydrogen peroxide are measured into the Bunsen bottle by a pipette. The blood is added by means of a pipette through the neck of the bottle, and the cork is quickly inserted. The pinch cock between the bottle and the burette is immediately released, all the bottles are placed in a shaker and the latter is run for 10 minutes at the rate of 275 shakes per minute. The evolution of gas is complete in this time and futher shaking adds nothing to the volume of oxygen released. Thus with each sample of blood we can obtain six determinations simultaneously under uniform conditions of temperature, pressure and agitation (see fig. 1). The results obtained in this way are fairly uniform but even under the conditions described there are variations usually between 0 and 3 per cent. In all cases it is desirable to dilute the blood until the total oxygen given off is less than 100 cc., otherwise the peroxide foams, bubbles are carried over into the burettes and the readings are less accurate.

We are aware of the fact that there is an error in the data in this paper because the release of oxygen took place at a variable room temperature instead of at some fixed uniform temperature for all the experiments. We are also fully aware of the fact that the amount of oxygen released by the same blood from different samples of peroxide varies between wide limits and that the amounts released from the same sample varies with age of the sample and with the reaction. In the results which follow in tabular form, no attempt was made to run all the bloods reported in this paper on a single sample of peroxide. All results assembled in a table with the exception of those in table 1 were run on a single sample of peroxide and at as nearly the same time as

the nature of the experiment would permit. Hence while the data given in the different tables cannot be compared directly, yet the figures assembled in a single table are in all cases comparable. The nature of the data here did not make absolute quantities necessary for the object was merely to compare the bloods under different conditions without reference to absolute catalytic power. We cannot compare bloods of different animals as regards *absolute* catalytic power, but we can say that $\frac{1}{15}$ cc. of cat's blood usually releases about the

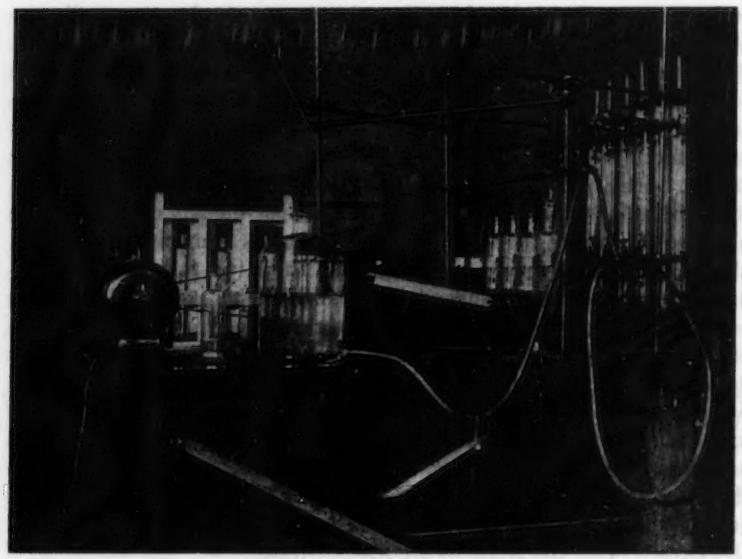

Fig. 1

same amount of oxygen as $\frac{1}{2}$ cc. of dog's blood from a single sample of hydrogen peroxide.

2. Normal variations. An idea of the various readings where repeated determinations were made on the same sample of blood is best secured from table 1.

It is evident from table 1 that the variations where a number of readings are taken are considerable, even where the same samples of blood and hydrogen peroxide are used. For that reason where differences of catalytic power are small in successive samples of blood, too much im-

portance must not be attached to these differences. This applies particularly to much of the past work, from which no idea can be formed from reading the article in regard to the number of determinations made on each sample of blood or tissue.

It is also evident from the data we have given that the amount of catalase present in the blood of animals of the same species varies within wide limits, as well as the fact that variations between species may be even wider. It is, therefore, self-evident that a comparison between the bloods of animals of different species with the idea of ob-

TABLE 1

Variations in oxygen released in simultaneous or successive tests in various animals

ANIMAL	DUPLICATE TESTS USING SAME SAMPLE OF BLOOD ON HYDROGEN PEROXIDE												
	1	2	3	4	5	6	7	8	9	Average	Corrected average at 0°C. 760 mm.	Amount used	Oxygen released by 1 cc. of blood
												cc.	*cc.*
Dog 1	47	47	47	47	47					47.00	44.14	½	88.28
Dog 2	21	20	22	21	21	21	21			21.00	19.24	½	38.48
Dog 3	13	13	11	12	12					12.20	11.78	½	23.56
Dog 4	79	81	82	80	80	80				80.33	74.53	½	149.06
Dog 5	61	63	64	61	64	64				62.66	59.01	½	118.02
Cat 1	92	93	96	93	96	94				94.00	79.88	1/15	1198.20
Cat 2	77	82	82	79	79	80	79	80	81	79.90	67.28	1/15	1019.20
Horse 1	67	67	66							66.60	64.89	1/20	1297.80
Sheep 1	19	19	19.5							19.00	18.90	1/10	189.00
Goat 1	100	101	94	91	93	94	98	101	93	96.10	88.99	½	177.98
Man 1	73	74	73							73.66	62.60	1/15	939.00
Man 2	56	54	57	57	55	54	54	57		55.50	50.85	1/15	762.60

taining an explanation of the reason why small animals oxidize more rapidly than large ones, is entirely impossible and obviously cannot give any information on the point at issue. Other factors besides mere weight or bulk are certainly at work, and size is only one and relatively an unimportant factor. The catalytic power of the blood of a dog is less than the catalytic power of the blood of a cat of the same weight—a fact which shows clearly the fact that size is only one of probably many factors.

3. Effect of thyroid feeding. We were, unfortunately from the standpoint of theory, not able to confirm the findings of Burge (5) regarding

the results in thyroid feeding. On this part of the subject we carried out three series of experiments; but since the work in the first two series gave such unsatisfactory results—because of wide discrepancies in reading on duplicate tests—we disregarded the results and report here only the data from a satisfactory series of three healthy animals.

The cats were under observation for a period of seven days before thyroid was administered. During this period the heart rate was taken daily and a count of red cells was made. On the seventh day the cats were bled from the jugulars without ether, and then about 15 grams of dry thyroid was administered with a stomach tube and was followed by 75 cc. of whole milk before the tube was removed from the esophagus. The administration of thyroid and of milk was repeated

TABLE 2

Effect of thyroid feeding

ANIMAL	BEFORE THYROID				AFTER THYROID				Total fed
	Weight	Average heart rate	Blood count (reds)	O_2	Weight	Average heart rate	Blood count (reds)	O_2	
				cc.				*cc.*	*grams*
Cat 23	6½	158.0*	8,460,000*	95.8	6	185.0†	9,495,000†	84.6	90
Cat 24	8¾	148.0*	7,930,000*	81.7	7¼	222.0‡	8,010,000‡	76.9	105
Cat 25	5⅝	183.0*	12,090,000*	169.9	5	186.0‡	9,060,000‡	102.5	105
Average		163.6	9,493,000	119.1		197.6	8,855,000	88.0	

* Average of 7 observations on successive days before thyroid feeding.
† Average of 5 observations on successive days during thyroid feeding.
‡ Average of 6 observations on successive days during thyroid feeding.

daily during the thyroid period, as well as the taking of the heart rate and the enumeration of erythrocytes. In addition all the cats were fed freely on milk and meat of which they took but little.

For the sake of brevity the results of the series have been condensed into table 2.

As can be seen from the table, all the animals lost weight in spite of the forced feeding; all had an increased heart rate, very slight in no. 25 but marked in the other two; all showed a *decrease* in the power of the blood to release oxygen from hydrogen peroxide, instead of an *increase* reported by Burge (4). In addition notes made at the time the experiments were carried out show that the animals suffered from a severe diarrhea. Thus in every case where the cat is fed thyroid up

to the point of the appearance of toxic symptoms, tachycardia, diarrhea and loss of weight, the amount of oxygen released by the blood is in every case less than normal. In our series the average amount released by $\frac{1}{15}$ cc. of blood was 119.1 cc. After thyroid feeding for five to six days the average amount released was 88.0 cc.

We are dealing here with a question of fact, and in favor of our results as compared with those reported by Burge we wish to point out possible errors which we avoided. First, our results are the results of averages, not single determinations. How many determinations he made on each blood cannot be determined from his articles. Ours are the average of nine determinations in each case. Second, in our series each cat constitutes his own control, a much better arrangement because of the wide variation between different animals in the same species. Third, the author referred to could not be certain in regard to the amount of thyroid the animals received for they took the substance mixed in their food. That the author experienced difficulty in getting the animals to eat thyroid is evident from his paper. That our cats received the amounts recorded is certain, that they retained it for at least thirty minutes was covered by continued observation after the drug was administered, that it was absorbed is evident from the toxic symptoms noted above. Fourth, Burge made no attempt to maintain the water content of the blood and thus did not avoid the possible complication of concentration of the blood due to a loss of water by normal excretion and decreased ingestion, or from increased elimination from the diarrhea, if his cats got enough thyroid to manifest toxic symptoms. This factor is important because the loss of water leads to concentration of the form elements, and to an enormous error; for as is known from earlier work (22) and confirmed by data in table 3, the catalases of blood are found in the form element, not in the fluid. We avoided this error by the fact that we administered 75 cc. of whole milk with the thyroid, and we believe this amount to be adequate to maintain the water balance in the blood of cats as large as the ones we employed in our experiments.

Further, it is interesting to note that not only is the amount of oxygen released by the blood reduced by thyroid feeding, but the number of corpuscles required to release a cubic centimeter of oxygen is increased and hence the amount of oxygen released by each corpuscle is reduced. This fact is brought out from the results recorded in the table just mentioned:

Number of corpuscles required to release 1 cc. of oxygen

	BEFORE THYROID	AFTER THYROID
Cat 23	5,887	7,444
Cat 24	6,510	7,100
Cat 25	4,744	5,892

It is possible to estimate the number of cubic centimeters of gas released by 1 corpuscle:

	BEFORE THYROID	AFTER THYROID
	cc.	*cc.*
Cat 23	0.000169	0.000134
Cat 24	0.000153	0.000140
Cat 25	0.000210	0.000169

From these facts it is clear that not only does thyroid feeding reduce the catalytic power of the blood as a whole, but it also reduces the amount of oxygen released from hydrogen peroxide by each individual corpuscle, in every case. The data here given do not include the leukocytes or platelets. Therefore the loss of weight from thyroid feeding cannot be due to an increased catalase content of blood, for on more accurate measurement *the catalase content of the blood is not increased but is decreased by feeding thyroid.*

Having studied the effect of thyroid feeding we considered it wise to study some of the simple facts regarding the catalytic power of the blood of animals. We selected cats because of the ease with which they could be secured and because of the high catalytic power of the blood. The blood was drawn under light ether anesthesia and thoroughly defibrinated by whipping. All the samples in the series recorded in tables 3 and 4 were run on the same hydrogen peroxide, and the series for each cat was completed on the same day the blood was drawn.

4. The relation of the catalase to the corpuscle. In table 3 we report the results from a series of eight healthy, normal cats in whose blood we studied the catalytic power of whole blood, serum, washed corpuscles and of blood saturated with carbon monoxide (illuminating gas).

This table emphasizes the fact already mentioned in the discussion of table 1; viz: the variation between two animals of the same species

TABLE 3

Effect of washing corpuscles, and of saturation of blood with carbon monoxide

ANIMAL	WHOLE BLOOD	WASHED CORPUSCLES	SERUM	WHOLE BLOOD SATURATED WITH C = O
Cat 1	25.68	26.76	0	23.06
Cat 2	26.72	23.92	0	23.36
Cat 3	26.07	23.43	0	26.07
Cat 4	14.22	12.39	0	12.08
Cat 5	13.65	12.55	0	13.66
Cat 6	16.22	12.27	0	12.89
Cat 7	16.26	12.48	0	15.07
Cat 8	19.75	17.50		18.81
Average	19.82	17.66	0	18.05

In each case 1 cc. of blood was diluted to 10 cc. with 0.9 per cent NaCl, and 1 cc. of the mixture (hence $\frac{1}{10}$ cc. of blood) was used as a test.

is enormous. Thus cat 2 released twice as much oxygen from the same hydrogen peroxide as cat 5; hence the variations amounted to 100 per cent between animals. Yet so far as we could determine both cats were nearly equally active or rather inactive as the readings were taken in the summer. Washing the corpuscles reduces the amount of oxygen released by about 10.8 per cent. We do not believe that this decrease is of much significance; probably the loss of catalytic power is due to loss of corpuscles during the process of washing. That there must be some loss is almost inevitable. That centrifugation is not the cause of destruction is shown by the fact that if blood is merely centrifugated and then shaken up without withdrawal of the supernatant fluid, the readings from the normal blood and centrifugated blood check within the limits of error:

NORMAL BLOOD	CENTRIFUGATED BLOOD	WASHED BLOOD
79.88	79.34	74.29
90.56	90.65	79.09
79.90	81.40	74.00

Of course these experiments do not eliminate a possible "complementing" action between serum and corpuscle.

We are therefore justified in the conclusion that centrifugating the blood does not impair the catalytic power. The third point brought

out is the fact that the catalytic power of the blood is confined to the corpuscle, for in none of these cases did the serum release oxygen from hydrogen peroxide. In other experiments serum up to 10 cc. was added to hydrogen peroxide but in no case was more than 2 cc. of O_2 released in ten minutes and in this case considerable red discoloration was evident. Thus the conclusion is warranted that the catalytic power of the blood is resident in the corpuscles and not in the serum. Fourth, saturation of the hemoglobin of the blood with carbon monoxide by exposing the diluted blood to illuminating gas reduces the catalytic power by 8.9 per cent in the series of experiments quoted. In cats 3 and 5 reported in the table release of oxygen by the blood was the same no matter whether the hemoglobin was saturated with oxygen or with carbon monoxide. We have many other cases in agreement with these examples:

	BEFORE	AFTER
Dog	42.07	40.71
	24.45	25.86
	28.10	28.10
Cat	90.55*	92.85*
	67.78*	67.00*
	95.83*	110.60*
	81.67*	94.54*

* This hydrogen peroxide was a different sample from that used in table 3.

Thus the average result from the total of 12 cats tested, in which the blood was saturated with gas after leaving the body the releasing power before saturation was 41.18 cc. and after gas 42.45 cc. of oxygen. Thus it is evident that while in our short series of cases saturation of blood with gas after it leaves the body reduces the catalytic power, this reduction is not evident in a larger series. In the series of three dogs cited the catalytic power before C = O saturation was 31.54, after saturation it was 31.54. These results are in accord with those of Jolles and Oppenheim (22), and opposed to those of Winternitz and Melloy (24). In all probability in the latter's case the change is due not to the direct action of the C = O, as they have assumed, but to some effect of gas upon the body other than the saturation of the hemoglobin with carbon monoxide. Fifth, the table shows the wide variation in the blood from apparently normal cats, there being 100

per cent variation between the highest and the lowest although the animals all seemed equally active and normal. The sluggish animals *did not* show a low, nor the active animals a high oxidase content as is claimed by Burge (13).

Further, dog 4 of table 5 was one of the most active dogs studied, yet his catalytic power was far below the average normal for all the dogs studied. There is absolutely no doubt in the mind of the author that there is *no relation* between the catalase content of the blood and the activity of the animal. The determinating factor as to whether an

TABLE 4

Effect of laking agents

	WATER LAKING	FREEZING AND THAWING	SAPONIN		ETHER		CHLOROFORM		ACETIC ACID		NaOH		NORMAL
			Laked	Control	Laked	Control	Laked	Control	Laked	Control	Laked	Control	
1	9.06	5.10	8.78	21.20	18.51	23.43			1.88	21.54	1.56	30.29	25.68
2	10.56	2.90	9.50	24.11	15.23	25.57	9.34	24.85	0.46	24.85	0.61	70.07	26.72
3	17.00	10.39	12.28	25.98	11.62	24.18	12.18	25.50	0.00	23.89	0.28	30.32	26.07
4	5.59	3.02	6.69	15.25	5.32	11.29	2.57	12.21	0.91	14.32	0.46	14.68	14.22
5	3.96	4.70	4.88	12.54	3.32	10.60	1.47	9.77	0.55	11.62	0.27	13.65	13.65
6	6.85	4.14	5.40	16.76	3.24	15.86	2.52	17.12	0.54	14.82	0.00	17.12	16.22
7	5.96	2.31	5.96	14.42	7.39	15.72	7.39	15.07	2.77	17.57	0.00	16.26	16.26
8	10.63	5.73	9.40	18.81	11.58	18.81	10.16	19.38	4.51	19.75	0.00	25.40	19.75
Av....	8.70	4.37	7.86	18.63	9.53	18.18	6.52	17.70	1.45	18.54	0.39	27.22	19.82

Each test was made by adding 1 cc. of a mixture of laked blood 1: 10 to 50 cc. of H_2O_2. Thus the figures represent the oxygen released from 50 cc. of H_2O_2 by $\frac{1}{10}$ cc. of blood. Isotonicity was secured in the case of water laking before testing the catalytic power. The control was made by adding the laking agent to the H_2O_2 before the blood was added.

individual is sluggish or alert would seem to depend not so much on the catalase content of the blood as upon the nervous system.

The next step in the subject was the determination as to what portion of the corpuscle is responsible for the release of oxygen from hydrogen peroxide. Upon this point information was obtained by laking experiments. Various forms of laking were employed as is shown in table 4. This table contains data from the same animals, made at the same time as the results cited in table 3.

It is evident from the table that laked blood does not release as much oxygen from the peroxide as does normal blood. Of the agencies em-

ployed, ether was least effective, and NaOH most effective in reducing the catalytic power. None of the substances employed modified the amount released to any very great degree as is shown by the comparison of the control with the normal amount released, except in the case of NaOH. This substance shows a favoring action, undoubtedly by the neutralization of the acid present in the U. S. P. preparation of hydrogen peroxide on the market. It is probable, judging from the colors produced, that the varying reduction of catalase content of the laked blood depends upon the degree of laking rather than upon fundamental differences in the processes of laking. Laking by water and by freezing and thawing are without doubt mechanical, yet these produce marked reductions in the catalytic power. If the presence of enzymes is the only factor concerned in the release of oxygen, it is strange that any process as mild as the mere introduction of water, or simply raising and lowering the temperature between 0°C. and 23°C. should produce such a marked reduction in the activity of the enzyme, for most enzymes withstand the action of water and wide ranges in temperature and readily withstand temperatures around 0°C. Thus it is evident that for normal catalytic activity the presence of the uninjured corpuscular membrane is essential. Whether the loss of catalytic activity is due to the destruction of the enzyme or to the loss of surface of the corpuscle is not clear. It has been impossible up to the present time for us to recover the enzyme, which is stated by Senter (25) to be in combination with the stroma. The laked blood—thus containing the mixture of hemoglobin and stroma—shows very little catalytic action and the addition of even large amounts of stroma—from as much as 10 cc. of blood for a single test—releases very little oxygen, 2 cc. Thus with the laking of the corpuscle the enzyme disappears from the blood and is lost, either because of destruction of the enzyme or because of a loss of surface.

5. Effect of anesthetics. Regarding the effect of anesthetics on the catalase content of blood, Burge concludes that the catalase content of the blood of animals is markedly reduced during the induction of anesthesia, and increased to above normal during recovery. The figures published were as follows:

	BEFORE	FOURTH STAGE	DECREASE	AFTER	INCREASE
			per cent		*per cent*
Dog 1	70	48	32	75	56
Dog 2	62	42	29	59	40
Cat 1	875	600	31		

One fundamental error in this work is self-evident: The normal was taken on animals so under the influence of morphine and ether that the insertion of a tracheal cannula was possible—an error inexcusable when the explanation of anesthesia is under consideration. Since animals were being studied in this laboratory under morphine and ether for another problem, it was considered advisable to subject the work on anesthetics to further tests, for this problem is of fundamental importance. The results are to be seen in table 5 where the data from ten experiments on six animals have been assembled.

As can be seen from the table the normal variation observed between individuals of the same species is very wide. The greatest catalytic activity observed was in dog 5, experiment A, whose blood released 66.22 cc. of oxygen. The lowest was dog 4, experiment B, whose blood released only 5.96 cc. of oxygen. Yet both animals were equally healthy and of the two dogs no. 4 was certainly the more lively animal. Thus we cannot credit the statement recently made (13) that it is possible to "pick out dogs with high catalase content on the basis of liveliness." As can be seen from the table, the average normal releasing power of the blood of dogs for oxygen amounts to 34.74 cc. for each $\frac{1}{2}$ cc. of dog blood. After morphine this average is reduced to 33.67 cc. Leaving out experiment C, dog 5, and experiment B, dog 6, in which there was obvious error due to imperfect defibrination of the blood, the average was 35.71 cc. After ether for fifteen minutes it is increased to 37.39 cc., after ether for one hour, or until the death of the animal, 36.92 cc. In only three of ten cases was there a decrease, and in two of these the animal was dead from an overdose of ether. It is, therefore, evident that under ether anesthesia there is no such reduction of catalytic power as is reported by Burge in his articles, (7), (8), (10), in which he states in his conclusion: "The catalase content of the blood decreases during the administration of ether and increases during the recovery from ether." We can offer no suggestions as to why our results differ from those reported by Burge, unless the difficulty lies in the method he employed, which we consider to be extremely inaccurate. However, that does not explain the gradual decrease and increase which he reports. If the errors lay entirely in the method such as we noted when employing his methods, we would expect irregular variations, not the uniform change which he reports.

Further, Burge (14) finds with alcohol an increase in the catalytic power of the blood, and this he believes to be the explanation of the beneficial results of alcohol in diabetes. As is well known, alcohol pro-

duces in large doses a condition objectively similar to anesthesia, yet according to Burge's measurements the catalases are increased. Are we to believe that the action of alcohol and ether is fundamentally dif-

TABLE 5

Effect of anesthetics

ANIMAL	DATE	CUBIC CENTIMETERS OF OXYGEN RELEASED FROM 50 CC. OF H_2O_2 IN TEN MINUTES BY ½ CC. OF BLOOD, WITH CONSTANT SHAKING								REMARKS
		Normal		After morphine, 30 minutes		After ether 15 minutes		After ether, 1 hr., or death from ether		
		Number of determinations	Average	Number of determinations	Average	Number of determinations	Average	Number of determinations	Average	
Dog 1.....	October 23	5	44.14	5	42.54	5	42.89	5	42.39	Dog dead of ether in 1 hour
Dog 2....	October 25	3	53.96	3	46.22	3	47.96	3	42.07	Dog dead of ether in ½ hour
Dog 3.....	October 26	6	19.24	3	18.32	6	22.60	6	23.74	After observing for 1 hour, dog killed with ether. Blood = 23.85
Dog 4A...	November 6	3	6.53	3	9.58	3	9.89	5	11.78	November 7 = 8.96 cc. November 8 = 9.00 cc.
Dog 4B...	November 9	3	5.96	3	6.27	3	6.92	3	7.21	November 10 = 7.45. November 13 = 4.45
Dog 5A...	November 6	3	66.22	3	69.43	3	70.66	6	74.53	
Dog 5B...	November 8	3	49.79	3	54.74	4	55.86	3	59.47	
Dog 5C...	November 9	3	53.05	3	32.13*	3	61.33	6	59.01	
Dog 6A...	November 8	3	23.82	3	22.27	3	30.42	3	26.77	
Dog 6B...	November 9	3	24.74	3	18.84*	3	25.43	3	22.28	
Average.....			34.74		35.71		37.39		36.92	

* Blood not properly defibrinated, numerous clots, and the estimations are too low. These figures are not included in the average of the column.

ferent? Further his premise that the pathology of diabetes is the failure of the animal organism to oxidize sugar because of decreased catalytic power of the tissue is not yet established.

Naturally since no decrease in catalytic power can be demonstrated, the theory of anesthesia he advocates is untenable.

6. Catalase of solid tissue. Our attempts to measure the catalytic power of solid tissue were unsatisfactory. How recent writers secure the uniform results they report is a mystery, to be explained only on the basis of a single test on each sample. If the method of determining catalases is reliable then results obtained by running a series of three to six tests of the same tissue against the same sample of hydrogen peroxide should give uniform results. Further, granting that the above test can be made satisfactorily, in comparing the same tissue from different animals or different tissues from the same animal where the tissues must be prepared separately, what is the criterion by which the fineness of division of the tissues prepared separately can be judged? It is very important that the state of division be the same in the sample to be compared for a finely ground sample will always release more oxygen from hydrogen peroxide than one less finely ground. Blood allowed to clot partially and then whipped so that fine clots are suspended yields less oxygen than blood well defibrinated. (See experiments 5C and 6B, table 5). This introduces the difficulty of preparing tissues from different sources, as for example heart muscle with skeletal muscle, a difficulty so great that a true comparison of the catalytic power of the two tissues has not yet been made.

We wish to present type examples of our results where a number of tests were made on a single carefully prepared tissue run against hydrogen peroxide. These tests involve five tissues only, viz., brain, heart, liver, intestines and skeletal muscles.

Experiments on brain. Young cat. Bled under ether. Aorta and carotid were perfused with warm salt solution. Brain and medulla were removed and ground in mortar for thirty minutes until a uniform, creamy consistency was secured. To 10 grams of brain tissue was added sufficient 0.9 per cent NaCl to make 100 cc. To this emulsion were added 12 large glass beads, and the vessel was shaken until a uniform emulsion was secured. Ten cubic centimeters of this emulsion, and thus 1 gram of brain tissue, were added to 50 cc. of hydrogen peroxide in each bottle, and all tests were run simultaneously for two hours. Readings were made at ten-minute intervals at first and longer intervals later. The results are recorded uncorrected in table 6. The tissue showed a tendency to clump and to rise to the surface during the early part of the shaking. Later the particles remained separate from each other.

Experiments on liver. Dog. Ether anesthesia. The animal was perfused through the aorta with warm salt until the liver was free from blood. The liver was ground in a sausage grinder and then triturated with a mortar and pestle. One gram of the ground tissue was accurately weighed into a watch crystal, washed

into the Bunsen bottle by 10 cc. of 0.9 per cent NaCl. Fifty cubic centimeters of hydrogen peroxide were added to each bottle and the tests were shaken as before noted. Readings were made as in the previous experiment. The results are recorded uncorrected in table 7. The tissue floated much of the time.

Experiments on heart tissue. The heart was removed under ether while still beating, and perfused backward through the aorta under about 1 meter pressure with warm salt. The heart beat vigorously during the early part of the perfusing and later was massaged freely until bloodless. The tissue was then ground in the sausage grinder and later triturated with a mortar and pestle until in a very

TABLE 6

Rate of the release of oxygen by 1 gram brain tissue

TIME	BURETTE					AVERAGE	ERROR
	1	2	3	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	76	68	72	72	72	72.0	5.50
20	90	80	84	91	84	85.8	7.25
60	99	97	90	99	95	96.0	6.20
90	104	113	90	107	112	105.2	14.40
120	104	132	90	107	113	109.2	20.90

TABLE 7

Rate of release of oxygen by 1 gram liver tissue

TIME	BURETTE			AVERAGE	ERROR
	1	2	3		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	203	165	162	176.6	14.8
20	265	219	218	234.0	13.2
30	314	261	265	280.0	12.1
40	378	319	324	340.3	9.00
50	415	361	358	378.0	9.80
70	519	463	478	486.6	6.63

fine state of division. The tissue tests were run as in the case of the liver. Results are recorded in table 8.

Experiments on intestine. Young cat. Ether anesthesia. Bled from the heart and later perfused with warm salt solution until the tissues were bloodless. The small intestines were removed and thoroughly cleansed of their contents. They were then slit longitudinally and the mucosa and part of the muscularis was scraped off into a mortar. The tissue was then carefully triturated with mortar and pestle. One gram by weight was tested against hydrogen peroxide, as in the previous tests, except that the peroxide had been neutralized with sodium carbonate. The results appear in table 9.

Experiments on skeletal muscle. Muscle was removed from the posterior aspect of the hind legs and lumbar region of the cat from which the intestines had been removed. The muscle was cut finely with the scissors and then ground with an equal amount of clean sand until the tissues were finely subdivided. One

TABLE 8

Rate of release of oxygen by 1 gram heart tissue

TIME	BURETTE					AVERAGE	ERROR
	1	2	2	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	39	46	39	51	50	45.0	13.3
20	43	48	46	54	55	49.2	12.6
30	44	48	49	55	56	50.4	12.7
40	45	48	51	56	57	51.6	12.7
50	45	48	53	58	60	53.0	15.0

TABLE 9

Rate of release of oxygen by 1 gram intestinal muscle and mucosa

TIME	BURETTE					AVERAGE	ERROR
	1	2	3	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	378	339	312	315	391	347.0	12.6
20	452	423	392	405	458	426.0	7.9
30	476	452	420	445	473	452.3	7.1
60	499	488	461	486	498	486.4	4.4

TABLE 10

Rate of release of oxygen by ½ gram of voluntary muscle

TIME	BURETTE					AVERAGE	ERROR
	1	2	3	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	126	119	118	124	127	122.8	3.9
20	166	164	191	194	186	180.8	9.2
30	180	175	209	211	205	196.0	10.7
60	196	192	227	232	227	214.8	10.6
90	198	193	232	236	231	218.0	11.4

gram of this mixture of muscle and sand, thus, 0.5 gram of voluntary muscle was tested against neutralized hydrogen peroxide. The results are shown in table 10. The sand above, added to hydrogen peroxide, released no oxygen in thirty minutes. Therefore, the reaction here recorded is due to the action of substances in the muscle.

From these experiments it is evident that when dealing with the solid tissues, even in a finely subdivided state, the evolution of gas is by no means completed in the ten minutes allowed by Burge, or in the fifteen minutes allowed by Alvarez and Starkweather (26), (27), (28), in determining the catalase content of solid tissue. In the case of the brain tissue four of five tests evolved some gas after sixty minutes, and the greatest deviation from the average of five tests varied from 5.5 to 20.9 per cent in the various readings. As can be seen in table 11, in one burette only 51 per cent of the total gas ultimately released was released in the first ten minutes; in another burette under identical conditions 80 per cent of the total gas ultimately released was released in the same time. Taking the average 65.9 per cent was released, and the greatest variation from this average was 14.9 per cent. It is therefore evident that for brain tissue a ten-minute test is absolutely inadequate.

TABLE 11

To show the percentage of the total oxygen released in ten minutes

TISSUE	BURETTE					AVERAGE	DIFFERENCE FROM AVERAGE
	1	2	3	4	5		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	73	51	80	67	64	65.9	14.9
Liver	39	35	33			36.2	3.2
Heart	86	95	73	88	83	84.9	11.9
Intestine	75	67	67	64	78	71.3	7.3
Voluntary muscle	63	61	51	52	55	56.3	5.3

As can be seen from table 12, in one burette only 60 per cent of the total gas ultimately released was released in the first twenty minutes; in another burette under identical conditions 93 per cent of the total gas ultimately released was released in the same time. Taking the average 77 per cent was released, and the greatest variation from this average was 17 per cent. It is therefore evident that for brain tissue a twenty-minute test is absolutely inadequate. In the case of the liver, all the tests were still evolving gas after seventy minutes of shaking. In fact some oxygen was evolved the next morning when the shaker was started after standing untouched all night. The greatest deviation from the average varied between 6.63 and 14.8 per cent. The details of error are to be seen in tables 11 and 12. In the case of the heart three of five tests were evolving gas after forty minutes of shaking and the deviation from the average varied between 12.6 and 15 per cent.

(See tables 11 and 12.) The writers referred to above record no long time experiments and it is thus self-evident that the results recorded by them measure not the total catalytic power of the tissue studied, but only a small and by no means necessarily equal fraction of the catalytic power of each tissue studied. That intestine and voluntary muscle are subject to the same error as the other solid tissues is evident from tables 9, 10, 11 and 12.

We believe that the work we have reported is proof that this method of studying catalase is unreliable, even when the same sample of tissue is used in duplicate tests, because the factor of error is from 5 to 20 per cent if we consider the variations between different tests. The factor of error in the solid tissues when we compare the amount of gas released

TABLE 12

To show the percentage of the total oxygen released in twenty minutes

TISSUE	BURETTE					AVERAGE	DIFFERENCE FROM AVERAGE
	1	2	3	4	5		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	86	60	93	67	63	77.0	17.0
Liver	51	47	45			48.0	3.0
Heart	95	100	86	93	91	92.8	7.2
Intestine	90	86	85	83	91	87.5	3.5
Voluntary muscle	83	85	83	82	80	82.9	2.9

in ten minutes in any single test with the total gas ultimately set free in the same test may vary anywhere from 3.2 per cent to 14.9 per cent from the average. If the reading is made after twenty minutes the factor of error varies between 2.9 per cent and 17 per cent from the average. The allowance made for either of these factors must be at least *doubled*, if tissue from different animals or different tissues from the same animal but prepared separately, are used, because of the utter impossibility of reducing all the tissue to the same state of subdivision. Thus it is with regret that we are forced to the conclusion that the data on the catalytic power of solid tissues prepared according to the methods employed are worthless, and the theoretical conclusions founded on these data are without the necessary foundations in fact.

SUMMARY

1. A method which measures the catalytic power of the blood is given accurate to within 5 per cent in most cases.

2. There is 1000 per cent variation in the bloods of different animals (dogs) under identical conditions, and wide variations in the bloods of animals of different species.

3. There is a reduction in the catalytic power of blood during thyroid feeding; this is true not only for the whole blood but for each corpuscle also. Thus increased catalytic power of the blood can not explain the loss of flesh during thyroid feeding, either because of the increased oxidation of material in the blood stream, or because of increased loss of catalase to the blood by the tissues, resulting in over-activity of the autolytic ferments, normally held in check by catalase.

4. The catalytic activity of the blood is confined to the corpuscle.

5. The catalytic power of the corpuscle is not influenced by the oxygen-carrying power of the blood.

6. There is a slight increase in the catalytic activity of blood during ether anesthesia, hence a decrease in the catalytic power of the blood cannot be the mechanism of action of the anesthetics.

7. No method has yet been devised which adequately measures the catalytic power of the solid organ. Oxygen is evolved for at least thirty minutes and the error is from 5 to 20 per cent.

CONCLUSION

Since the catalytic power of the blood varies between such enormously wide limits, 100 per cent to 1000 per cent, in animals of the same species under the same conditions, we do not believe that the catalases can be particularly important and hence do not believe that a study of catalases can possibly explain the mysteries of the processes of oxidation.

(The writer wishes to acknowledge the help of Dr. E. L. Ross in the development of the apparatus, and of Messrs. Eymundson, Greenwood, Hubbard and Schlosser in carrying out the experiments.)

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THE INFLUENCE OF INTERNAL SECRETIONS ON THE FORMATION OF BILE

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The experiments that form the basis of the present report were undertaken in an effort to determine the effect of certain of the glands of internal secretion upon the output of bile by the hepatic cells. For our study we selected the following gland substances: mammary, orchic, ovarian, pancreatic, splenic, thymic and thyroid. These were all obtained from Armour & Company. To the foregoing list we added solution of adrenalin chloride, prepared by Parke, Davis & Company, and secretin, prepared by the method we have described in connection with other experiments (1).

The procedure was the same in all of the experiments: The animal to be operated upon, usually a dog but in a few cases a cat, was anesthetized by morphine and ether, if a dog, or by ether alone, if a cat; a cannula was inserted in the left carotid artery and connection made with a mercury manometer in order to record blood pressure; another cannula was inserted in the right external jugular vein for the injection of a solution of the gland substance being studied. The abdomen was opened in the linea alba and a third cannula, specially constructed, was tied in the common bile duct; the cystic duct was now clamped or ligated, the edges of the abdominal incision brought together, and a towel wrung out in physiological saline solution at a temperature of 40°C. placed upon the abdomen so as to cover the wound completely. This towel was kept moist. Rectal temperature was taken from the time the anesthetic was started until the end of the experiment. An electrically heated operating table was used.

When all was in readiness an observer, who had no other duty, began to count the drops of bile as they fell from the end of the cannula tied in the common duct. All counts were made for an arbitrary period of twenty minutes. The first count was made before any agent was injected. When this initial count was finished the gland substance to be

studied was injected per jugular. In every case, except the experiments with adrenalin, a dose of 10 mgm. of the gland substance per kilogram of body weight of the animal was dissolved in 100 cc. of physiological saline solution, warmed to 37°C. on a water bath, and injected by means of a burette. The counting of the drops of bile was continued for a second twenty-minute period beginning at the moment the injection of the gland substance started. In most instances another twenty-minute count was made, giving an initial count with which comparison could be made and one or two counts after the gland substance had been administered.

No attempt was made to analyze the bile at any time during the course of any experiment so that we are unable to speak with regard to qualitative changes that may have resulted. The number of drops of bile secreted in each period may be regarded as indicative of the activity of the liver cells so far as bile production is concerned during that particular time and it was to this that our inquiry was directed.

The results of all the experiments have been arranged in groups, each group comprising the determinations made with one gland substance, and these are presented in the accompanying tables.

Adrenalin (table 1) produced a decrease during the first twenty minutes in every case. There were three occasions on which it was possible to make a second count after the injection and in two of these the number of drops of bile secreted was still less than during the period of the initial count.

Eight determinations were made with mammary gland substance (table 2); five showed a decrease from the initial count during the first twenty-minute period, two an increase and one no change. Where a second count was made after the injection, which was done in seven of the experiments, no definite trend was observable. It would seem that the effect of mammary gland substance on the secretion of bile is a temporary decrease.

Orchic substance (table 3) caused a lessened secretion of bile during the first twenty minutes after the injection in five out of seven experiments; in four of the five the effect was progressive, a further decrease occurring in the second twenty minutes.

In the five tests made with ovarian substance (table 4) there was a decrease in every instance, averaging 44.59 per cent in the first twenty minutes, and 59.36 per cent in the second twenty minutes.

Pancreas (table 5) was injected into nine dogs and the count of the drops of bile during the succeeding twenty minutes revealed a decrease

TABLE 1

Adrenalin. Dose: 0.1 cc. of a 1: 1,000 solution adrenalin chloride per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
7 (cat)	10	4	−60.00	5	−50.00
13	140	49	−65.00	No count	
27	9	8	−11.11	13	+44.44
47	16	12	−25.00	9	−43.75
60	13	10	−23.07	No count	

TABLE 2

Mammary substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
8	3	2	−33.33	2	− 33.33
11	46	23	−50.00	No count	
16	18	5	−72.22	2	− 88.88
25	5	5	00.00	15	+200.00
34 (cat)	6	7	+16.66	10	+ 66.66
46	15	12	−20.00	22	+ 46.66
49 (cat)	10	15	+50.00	14	+ 40.00
57	22	13	−40.90	5	− 77.27

TABLE 3

Orchic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
4	84	45	−46.42	23	−72.61
18	31	3	−90.32	35	+12.90
19	134	15	−88.80	9	−93.20
41	35	12	−65.71	4	−88.57
42	9	8	−11.11	4	−55.55
55	12	25	+108.33	No count	
62	25	35	+40.00	No count	

TABLE 4

Ovarian substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
5	19	18	−5.26	10	−47.36
20	35	8	−77.14	4	−88.57
43	4	1	−75.00	3	−25.00
56	30	27	−10.00	15	−50.00
59	9	4	−55.55	1	−88.88

TABLE 5

Pancreatic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
1	16	3	−81.25	3	−81.25
14	89	60	−32.58	42	−52.80
22	35	15	−57.11	No count	
23	121	108	−10.74	73	−39.66
30	3	2	−33.33	2	−33.33
38	42	22	−47.61	25	−40.47
48	8	3	−62.50	3	−62.50
50	25	17	−32.00	25	00.00
52	25	12	−52.00	No count	

TABLE 6

Secretin. Dose: 10 mgm. of a dried acid extract per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
9	31	35	+12.90	36	+16.12
10	1	6	+500.00	4	+300.00
15	2	6	+200.00	36	+1700.00
26	1	6	+500.00	1	00.00
35	36	64	+77.77	55	+52.77
36	4	18	+350.00	No count	
54	2	3	+50.00	No count	

TABLE 7

Splenic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
2	198	198	00.00	207	+4.54
21	89	45	−49.43	30	−66.29
24	4	8	+100.00	6	+50.00
31	207	205	−0.96	No count	
44	36	19	−47.22	20	−44.44
45	6	7	+16.66	6	00.00
61	37	18	−51.35	7	−81.08

TABLE 8

Thymic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
3	55	18	−67.27	61	+10.90
29 (cat)	42	38	−9.52	No count	
32	37	17	−54.05	13	−64.86
39	45	17	−62.22	31	−31.11
53	31	7	−77.41	2	−93.54

TABLE 9

Thyroid substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
6	8	10	+25.00	6	−25.00
12	24	13	−45.83	17	−29.16
17	20	11	−45.00	9	−55.00
28	3	10	+233.33	6	+100.00
33	6	5	−16.66	3	−50.00
37	17	15	−11.76	16	− 5.88
40	9	12	+33.33	8	−11.11
51	16	22	+37.50	No count	
58	8	4	−50.00	No count	

in all. The average decrease was 45.45 per cent. A further count was made in seven of these experiments and showed the effect to be maintained in all but one. The average decrease for this period below the initial count was 44.28 per cent.

In the seven cases in which secretin (table 6) was administered intravenously there was a marked augmentation in the amount of bile secreted. This increase averaged 241.52 per cent. A subsequent count was made on five occasions and in four of these the output of bile was still above the original level. Experiment 15 of this group is particularly striking; during the second period after the injection the amount of bile formed was six times as great as that during the first period and eighteen times that produced in the twenty minutes immediately preceding the giving of the secretin. The effect of secretin on this group of dogs is further confirmation of the results reported by Bayliss and Starling (2).

Splenic substance (table 7) was administered to seven animals and failed to exhibit any constant effect.

Thymus gland substance (table 8) uniformly brought about a decrease in the quantity of the biliary secretion during the first period after it was injected. This effect was still evident in three out of four experiments in which a second count after injection was made.

Intravenous injection of the substance of the thyroid gland (table 9), like splenic substance, was without constant effect.

A review of the literature has failed to reveal the report of any definite experiments with regard to the influence of the internal secretions on the amount of bile secreted. In a study of bile pigment metabolism Hooper and Whipple (3) state that in two groups of dogs, the members of one group having undergone splenectomy and those of the other group serving as controls, the quantity of bile pigment and of bile produced was identical. This result is in accord with our finding that splenic substance intravenously did not alter definitely the output of bile.

CONCLUSIONS

1. The amount of bile secreted is increased by secretin.
2. The amount of bile secreted is decreased by adrenalin and by mammary, orchic, ovarian, pancreatic and thymic gland substances.
3. The amount of bile secreted is not affected in a constant or definite manner by the substance of the spleen and thyroid gland.

We wish to acknowledge assistance rendered in the conduct of these experiments by Dr. George Hays and Messrs. L. and M. Notkin.

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BAKERS' YEAST AS FOOD FOR MAN[1]

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That brewers' yeast possesses food properties wsa demonstrated long ago. However it was not until the stress of war seriously reduced the available food supply of the world that the attention of scientists was directed to the yeast plant as a possible source of food protein. The Germans were the first to make a comprehensive study of its nutritive possibilities. Such investigators as Rubner (1), Schottelius (2), Wintz (3), Winckel (4), Fuchs (5), Kellner (6) and Völtz (7) testify to the dietetic importance of brewers' yeast, whereas Schrumpf (8) and others do not think it possesses much value as a food. Fendler and Borinski (9) are inclined to lay the difference in opinion of the above experimenters to the fact that the brewers' yeast is so variable in composition as to render impossible the comparison of data obtained from the use of yeast from different sources.

That the protein of yeast constitutes a suitable food for man is borne out by the investigation of its hydrolysis products (10). Such important amino acids as the following have been shown to be present: glycocoll, alanine, valine, leucine, phenylalanine, tyrosine, proline, aspartic acid, glutamic acid, tryptophan, lysine, arginine and histidine.

The yeast used by us in our studies possessed the following composition calculated on a water-free basis.

	per cent
Protein	52.41
Fat	1.72
Carbohydrate	37.13
Ash	8.74

The composition of the ash was as follows:

[1] The expense of this investigation was defrayed in part by a grant from Mrs. M. H. Henderson.

Phosphorus (P_2O_5)	54.5
Silicon (SiO_2)	1.2
Calcium (CaO)	1.4
Magnesium (MgO)	5.2
Sulphur (SO_3)	0.5
Potassium (K_2O)	36.5
Sodium (Na_2O)	0.7
Chlorine	trace
Iron	trace

It is apparent therefore that this dried yeast has at least three times the calorific value of beef of moderate fat content (11). That dried yeast is readily digested and utilized by the animal organism has been shown by Rubner (12), Fuchs (13), Kellner (14) and others. According to Völtz (15) yeast is one of the most digestible of feeding stuffs and possesses excellent dietetic properties.

So far as metabolism experiments are concerned very few are on record. The most comprehensive of these was carried out by Funk, Lyle and McCaskey (16). The most important remaining ones were reported by Prausnitz and Menicanti (17) and Thomas (18). Funk and associates experimented upon four men who were fed a diet in which yeast[2] was the *sole source* of protein. They report that "a large part of the yeast nitrogen apparently has no food value" and that "it is badly assimilated" but conclude with the statement, "in the field of nutrition research our studies have by no means enabled us to pronounce a verdict that yeast possesses no value in dietetics."

In our own experiments which we report on the following pages, we studied the metabolism of six men and found that four of the six showed an improved nitrogen balance when fed the yeast diet. Moreover when all of our data on the six subjects were reviewed we found that the yeast diet had produced an average daily gain of about 0.4 gram of nitrogen per man, above that noted before yeast was fed. The only interpretation is that these men found yeast a satisfactory article of diet. It seems to us that there are several factors which serve to explain the diametrically opposed conclusions of Funk and ourselves. In the first place in our tests we fed the same amount of nitrogen per day throughout the normal periods and yeast periods, whereas the amount of nitrogen fed by Funk during the normal periods was always much higher than that fed during the yeast period. In the second place, Funk used yeast as the *sole source of protein*, whereas we substituted a certain amount

[2] Obtained from American Pure Yeast Company of New York.

(9 per cent to 29 per cent) of the nitrogen of the normal diet by yeast nitrogen. In the third place, we used three types of diet while only one type was used in the other investigation. In the fourth place, Funk states that "the bad utilization of yeast may be partially due to the fact that the yeast had a disagreeable taste and was unpleasant to eat for any length of time." We avoided this feature by incorporating our yeast in wheat biscuits and in this way produced an yeast-wheat combination which had a most agreeable flavor. In fact the biscuits tasted better with the yeast than without it. And in the fifth place the kind of yeast used in the two studies was not the same, since Funk used a product furnished by the American Pure Yeast Company whereas we employed the ordinary bakers' yeast of commerce (Fleischmann). We feel that the divergence in the reports of Funk and ourselves was brought about by the influence of one or more of the above five factors.

TABLE 1

Approximate amounts of nitrogen ingested in the form of yeast (expressed as percentages of ingested nitrogen)

	EXPERIMENT					
	1		2		3	
Subject	F.	Sm.	H.	Sr.	G.	M.
Per cent	27	29	12	9	18	22

Preliminary baking tests. Before starting the experiments planned to study the influence of yeast upon the nutrition of man, it was necessary to make some preliminary baking tests in order to determine the influence upon the taste and physical characteristics of the loaf brought about by adding dried yeast to the flour used in bread making. These tests demonstrated that we could replace as high as 20 per cent of the flour by dry yeast and produce a loaf excellent in every way and possessed of an attractive flavor. This is equivalent to the replacement of 50 per cent of the protein of the flour.

In the various tests on men, from 9 per cent to 29 per cent of the nitrogen of the daily diet was replaced by this dried yeast (see table 1). The yeast was dried at 105°C. in a current of air and then put through proper milling processes to produce an yeast flour of the approximate fineness of ordinary wheat flour. The yeast flour mixed readily with the wheat flour, making a good homogeneous preparation. Excellent biscuits were prepared from this wheat-yeast flour.

We also made some tests upon the addition of yeast to meat preparations. In this connection we found that we could replace 25 per cent of the meat protein by yeast protein and have a nutritious and palatable mixture.

Experimental plan. The investigation was divided into three separate experiments, in each of which a study was made of the metabolism of two men. Each of these three experiments was again subdivided into from two to four individual experimental periods. In other words, a total of six different subjects was used in the three experiments and a total of eighteen periods was studied, the periods with two exceptions being seven days in length. All of the subjects were good reliable men who were thoroughly interested in both the scientific and practical aspects of the investigation.

In the first two periods of the *first experiment*, the men were fed a *high protein defective diet.* That is, they were given plenty of protein in the form of meat, wheat, etc. but the diet was not a *complete diet* from a strict nutrition viewpoint because it was lacking in the *indispensable water-soluble vitamine*, which is present in yeast and certain other foods. In the third period, the diet was supplemented by a small amount of yeast, (0.2 gram of yeast nitrogen) in order to add the essential vitamine just mentioned, and in the fourth period, a considerable part (27 per cent to 29 per cent) of the protein of the diet was replaced by yeast protein. In this period each subject received 8.43 grams of yeast nitrogen per day.

By the above procedure, the third period enabled us to study the value of the yeast vitamine when added in small amount to a defective diet, whereas period four enabled us to compare the digestibility or so-called "utilization" of yeast protein with the digestibility or "utilization" of proteins such as those of meat and wheat, which are customarily included in the diet of man.

The diet as fed consisted of meat, wheat biscuits, jelly, margarine, potato chips, crisco, corn starch, agar agar and water.

The actual cooking of the food was carried out by the head dietitian of Jefferson Hospital,[3] and accurately weighed amounts were fed to each man. Samples of all foods served were taken for chemical analysis and the analyses were always made in duplicate and when it was deemed necessary triplicate determinations were carried out. The urine was analyzed in twenty-four-hour periods. The feces were collected in

[3] We are under obligation to Miss Gladwin for her aid in this connection.

thymolized receptables and frozen. Samples representing the individual periods were analyzed.

The *second experiment* was planned to study the effect of yeast when added to a *low protein defective diet.* The diet was defective in that the "water-soluble vitamine" present in yeast and certain other foods was not included in sufficient amount.

The periods of "small" and "large" yeast addition were similar to those of the first yeast experiment. Here, however, only from 9 per cent to 12 per cent of the ingested nitrogen was in the form of yeast nitrogen. The urine and feces were collected and analyzed as already explained.

In the *third experiment,* the subjects were fed a normal complete diet, i.e., a diet which contained satisfactory protein, fat, carbohydrates, energy, inorganic salts and vitamines. The water-soluble vitamine was furnished by the use of *milk,* a food not included in the diet of the subjects of the first and second experiments.

In the period of large yeast ingestion in this test from 18 per cent to 22 per cent of the protein of the diet was replaced by the yeast protein.

For data regarding the nitrogen content of food, feces and urine of the three experiments, see table 2.

Discussion of the first experiment. In this experiment subjects "F" and "Sm" were employed. It being our desire to study the effect of yeast when added to and substituted in a high protein defective diet, we started the men on a diet containing over 16 grams of nitrogen per day (see table 2). This diet was so far in excess of the nutritional needs of the men that there was a daily gain of over 3 grams of nitrogen to the body. For this reason we ran an extra period in which the diet was cut to about 14 grams of nitrogen per day. The ingestion of this diet showed a daily gain of only 1.04 gram for "F" and one of 1.96 gram for "Sm."

With the above nitrogen balances as a basis we added a small amount of yeast to the diet. Each subject received only 0.24 gram of yeast nitrogen in a total daily nitrogen intake of about 14 grams. On this diet "F" gained 1.79 gram of nitrogen per day whereas "Sm" showed a daily gain of 1.67 gram.

In the final period of the experiment a considerable part of the protein of the diet was made up of yeast protein. The specially baked yeast biscuits, already mentioned, were used and from 27 per cent to 29 per cent of the ingested nitrogen was in the form of the nitrogen of yeast.

TABLE 2

Nitrogen balances

EXPERIMENT NUMBER	SUBJECT	NO YEAST PERIOD							NO YEAST PERIOD							SMALL AMOUNT OF YEAST PERIOD							LARGE AMOUNT OF YEAST PERIOD						
		Number of days	Nitrogen							Number of days	Nitrogen					Number of days	Nitrogen						Number of days	Nitrogen					
			Income	Outgo		Balance					Income	Outgo		Balance			Income	Outgo		Balance				Income	Outgo		Balance		
				Urine	Feces	Period	Daily					Urine	Feces	Period	Daily			Urine	Feces	Period	Daily				Urine	Feces	Period	Daily	
			grams	*grams*	*grams*	*grams*	*grams*			*grams*	*grams*	*grams*	*grams*	*grams*	*grams*		*grams*	*grams*	*grams*	*grams*	*grams*			*grams*	*grams*	*grams*	*grams*	*grams*	
1	F.	7	114.2	81.96	9.31	+21.9	+3.13	5	72.7	58.70	8.80	+5.2	+1.04	7	103.4	81.31	9.61	+12.5	+1.79	7	106.1	74.39	14.58	+17.1	+2.44
				91.3						67.5						90.9						89.0						
	Sm.	7	114.2	77.89	9.81	+26.5	+2.78	5	68.7	50.77	7.59	+9.8	+1.96	7	97.2	76.00	9.45	+11.7	+1.67	7	99.8	71.36	17.71	+10.7	+1.35
				87.7						58.4						85.4						89.1						
2	H.	7	74.6	60.31	6.78	+7.5	+1.07	7	58.7	46.75	7.48	+4.5	+0.64	7	59.8	46.49	7.18	+6.1	+0.87	7	59.2	46.14	8.92	+4.1	+0.58
				67.1						54.2						53.7						55.1						
	Sr.	7	74.5	68.47	10.68	−4.7	−0.67							7	75.9	69.11	11.27	−4.5	−0.64	7	75.3	62.95	10.06	+2.3	+0.33
				79.2												80.4						73.0						
3	G.	7	83.0	74.66	10.97	−2.6	-0.37													7	84.6	71.10	14.81	−1.3	−0.19
				85.6																		85.9						
	M.	6	60.	49.54	9.90	+0.8	+0.13													7	68.3	55.62	13.67	−1.0	−0.14
				59.4																		69.3						

In the case of "F" there was a gain of 2.44 grams of nitrogen per day, as compared with a gain of only 1.04 gram during the period when no yeast was included in the diet (see table 2). In other words, when the nitrogen of the wheat and meat of the diet was substituted to the extent of 27 per cent by the nitrogen of yeast, the body was better nourished as shown by the gain of 2.44 grams of nitrogen per day. This more desirable condition was brought about in the case of "F" in spite of the fact that the yeast was given in such large amount as to produce a mild diarrhea. The stools were very soft and the daily output of fecal matter was increased to 992.1 grams from a value of only 637.6 grams for the period during which no yeast was eaten. This experiment thus furnished a very nice illustration of the laxative action of bakers' yeast, an action which we had previously demonstrated upon invalids (19).

Subject "Sm" showed a more pronounced diarrhea than did "F" and his nitrogen balance showed a daily gain of 1.53 gram. There is no question but what both of these subjects would have shown higher gains of nitrogen had not the laxative action of the yeast produced an excessive output of soft feces. That the utilization values and the figures in the nitrogen balance sheet do not necessarily run parallel is brought out nicely in the experiment on "F," as shown in table 3. In the first period when no yeast was included in the diet 87.9 per cent of the nitrogen of his food was absorbed from the bowel and 1.04 gram retained by the body. When a small amount of yeast was added to the diet he utilized more of the food nitrogen (90.7 per cent) and also retained more (1.79 gram). But the most remarkable demonstration came in the period in which a large amount of yeast was eaten. Here only 86.2 per cent of the food nitrogen was absorbed from the bowel *but the significant fact is that 2.44 grams of it was retained.*

The food eaten by "F" during the second "no yeast" period contained 72.7 grams of nitrogen (table 2). Of this amount 8.8 grams were lost in the feces, leaving 63.9 grams as absorbed. Of this 63.9 grams only 5.2 grams were retained in the body. In contrast to this condition we find that 17.1 grams out of total absorption of 91.52 grams were retained during the "large yeast" period. By calculating on the basis of the available data just cited, it will be found that 8.1 per cent of the nitrogen which was absorbed from the intestine in the "no yeast" period was retained in the body of "F" whereas 18.6 per cent of the absorbed nitrogen was retained when this absorbed nitrogen was more largely made up of the nitrogen of yeast. It seems, therefore, when the diet contained considerable yeast nitrogen that the body made better

use of the food nitrogen than when no yeast was present. It is apparent that the yeast nitrogen was more satisfactory for the uses of "F" than was the nitrogen in the form of meat, wheat and the other foods used. The low utilization value (86.2 per cent) for "F" during the period of high yeast ingestion was due, as before stated, to the fact that the yeast had a pronounced laxative effect.

Subject "Sm" utilized 88.8 per cent of the nitrogen of his diet when no yeast was present whereas the utilization value rose to 90.3 per cent when a little yeast was added (table 3). In the high yeast period which

TABLE 3

Summary of nitrogen balances and utilizations

EXPERIMENT NUMBER	SUBJECT		NO YEAST	SMALL AMOUNT OF YEAST	LARGE AMOUNT OF YEAST
1	F.	Daily balance (grams)	+1.04	+1.79	+2.44
		Utilization (per cent)	87.9	90.7	86.2
	Sm.	Daily balance (grams)	+1.96	+1.67	+1.53
		Utilization (per cent)	88.8	90.3	82.3
2	H.	Daily balance (grams)	+0.64	+0.87	+0.58
		Utilization (per cent)	87.2	87.9	84.9
	Sr.	Daily balance (grams)	−0.67	−0.64	+0.33
		Utilization (per cent)	85.7	85.3	86.7
3	G.	Daily balance (grams)	−0.37		−0.19
		Utilization (per cent)	86.8		82.5
	M.	Daily balance (grams)	+0.13		−0.14
		Utilization (per cent)	83.5		80.0

followed there was a low utilization value (82.3 per cent) due to the laxative action of the yeast as already noted. For example, 872.5 grams of feces were passed during this period as against 496.4 grams when no yeast was present in the diet. Of course the bulk of this excess weight was water, as the stools were very soft. However, sufficient nutritive material was carried out of the bowel in the fluid mass to lower the utilization value appreciably.

It is quite significant that in spite of the large loss of water from the body in the soft feces, "F" *gained three pounds in body weight* on the

yeast diet and "Sm" gained one pound. Schottelius (20) reports gains in body weight by five out of nine subjects after yeast ingestion.

Discussion of the second experiment. In this experiment we studied the influence of yeast when added to and substituted in a low protein defective diet. The men used in these tests were "H" and "Sr." We started "H" out upon a diet containing only a little over 10 grams of nitrogen per day (see table 2) and notwithstanding this very low food intake his body retained 1.07 gram of nitrogen per day. We then ran an extra period in which the nitrogen content of the diet of "H" was cut to a little over 8 grams per day.

On this very low diet "H" gained 0.64 gram of nitrogen per day whereas "Sr" lost 0.67 gram of nitrogen per day on a diet containing 10.64 grams of nitrogen.

When a small amount of yeast was added to the diet the nitrogen balance sheets (tables 2 and 3) show that "H" retained more nitrogen (0.87 gram) than when no yeast was present. The nitrogen retention of the high yeast period was about the same (0.58 gram) as that for the period in which no yeast was eaten.

Subject "Sr" was losing nitrogen (0.67 gram daily) at the time the small amount of yeast was added to his food. There was a smaller loss of nitrogen per day (0.64 gram) after the yeast was added, but the difference was not significant. However, when the diet was so changed that yeast nitrogen was substituted for 9 per cent of the nitrogen of the meat and wheat of the diet, the balance very quickly shifted from a negative to a positive one. In other words, the data show that the body *lost* 0.67 gram of nitrogen every day for a week when no yeast was present in the diet whereas there was a *daily gain* of 0.33 gram of nitrogen for the period in which the large amount of yeast was eaten.

In the case of "Sr" the utilization values run parallel with the nitrogen balances. In the "no yeast" and "small yeast" periods a little over 85 per cent of the nitrogen of the diet was absorbed from the bowel while 86.7 per cent of the food nitrogen was absorbed when 9 per cent of yeast nitrogen was present. We thus see that when the diet had a high yeast value that the body of "Sr" showed better absorption from the bowel and likewise a higher retention of nitrogen by the cells of the body. It is interesting to note that "Sr" was one of the "furunculosis" patients in our therapeutic tests (21). In the case of "boils" the yeast produced a rapid and complete cure.

Although "H" was ingesting less food than "Sr," yet the percentage of the food nitrogen which was in the form of yeast nitrogen was 12

per cent in the high yeast period as against 9 per cent for "Sr." The utilization figure for this period was 84.9 per cent as against 87.2 per cent for the "no yeast" period and 87.9 per cent for the period of "small yeast" intake. The nitrogen balance for the "high yeast" period was about the same as that for the "no yeast" period.

The yeast ingestion was not sufficiently high in this experiment to produce the laxative effect noted in the first experiment.

Discussion of the third experiment. In this experiment we studied the influence of yeast when substituted for protein from other sources in a diet entirely complete and satisfactory in every way. Only two periods were run inasmuch as the period of "small yeast" was not necessary as the water-soluble vitamine was already present in the milk of the diet. The subjects of the experiments were "G" and "M."

Subject "G" was fed a diet containing about 12 grams of nitrogen (see table 2). On this diet he showed a negative nitrogen balance of 0.37 gram per day when no yeast was present in the diet. In the next period 18 per cent of the nitrogen in the form of wheat and meat was replaced by yeast nitrogen. A week on this diet yielded a minus balance of 0.19 gram per day. Apparently the yeast was as satisfactory for the nutritional uses of this man as was beef and wheat, notwithstanding the fact that the utilization value of the yeast period (82.5 per cent) was lower than that of the "no yeast" interval.

The diet of "M" contained about 10 grams of nitrogen (see table 2). On this diet he gained 0.13 gram of nitrogen per day and lost a similar quantity (0.14 gram) when 22 per cent of the nitrogen of this diet was in the form of yeast. There was also a somewhat lower utilization value (80.0 per cent) than when no yeast was eaten (83.5 per cent). Inasmuch as the diet of subjects "G" and "M" was perfectly complete from the nutritional standpoint without the addition of yeast it is not surprising that the yeast did not cause an increased nitrogen retention. No laxative action was observed in the case of the subjects of this experiment.

CONCLUSIONS

Bakers' compressed yeast forms a very satisfactory article of diet for man.

The yeast may be incorporated with meats such as hamburg steak and yields a preparation of very satisfactory taste.

If the yeast be dried in a current of air at 105°C. and pulverized it may be substituted for 20 per cent of the wheat flour used in bread making and yields a product which is very nutritious and of an attractive flavor.

From 10 to 30 per cent of the nitrogen of an ordinary mixed diet may be replaced by yeast nitrogen in the form of compressed yeast without detriment to the best nutritive interests of the individual.

We have demonstrated that the nitrogen of yeast is preferred by the nutritional mechanism of certain individuals, to the nitrogen in the form of such staple foods as meat and wheat. In the case of certain other individuals no differentiation is possible between the nutritive value of yeast and the staple foods mentioned.

The average individual may ingest yeast sufficient to yield from 1 to 2 grams of nitrogen per day, without securing any laxative effect. When as high as 4 grams of yeast nitrogen are eaten, a laxative effect is generally observed and in some cases soft diarrheal stools result. This laxative action of yeast may be put to good use in overcoming constipation.

When we take into consideration the experiments on all six of the men who served as subjects, we find that four of the six found the diet more satisfactory when yeast was included. In other words, subjects "F," "H," "Sr" and "G" showed an improved balance when yeast was present in the diet, whereas the differences in the balances of "M" are not significant and the variation in the balances of "Sm" is due to the fact that the subject was fed such a large amount (29 per cent) of yeast as to cause the passage of watery stools.

When all of the experimental periods are reviewed we find that the average daily gain of nitrogen when no yeast was present in the diet was 0.48 gram, whereas this gain was increased to 0.82 gram when yeast was eaten along with other foods.

Of the six subjects who ate the yeast diet, four made gains of from ½ pound to 3 pounds in body weight, in a period of two weeks, while the other two men showed a loss.

The relation of yeast to the growth of the animal body will be discussed in a subsequent paper.

The authors are grateful to Dr. Olaf Bergeim and to the subjects of the experiment for assistance given them.

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COMPRESSED YEAST AS FOOD FOR THE GROWING ORGANISM[1]

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Various experiments (1) made in this country and abroad have demonstrated beyond question the importance of so-called accessory food substances or vitamines in the growth-producing dietary. One of the earliest of these to be demonstrated was the vitamines of yeast (2). Later Funk, Funk and Macallum and Osborne and Mendel directed our attention anew to this important property of yeast. In each instance the above workers used brewers' yeast.

In our own experiments, which we herewith report, the ordinary household compressed yeast (Fleischmann) was utilized and was demonstrated to have very important growth-promoting properties.

DESCRIPTION

Experimental animals. Thirty-two white rats, ranging from 30 to 40 days of age were purchased from the white rat colony of the Wistar Institute. The thirty-two rats were included in six litters and were evenly divided as to sex, sixteen being males and a like number females.

For experimental purposes we divided the rats into four groups. Males and females were kept separate. Our experiments therefore entailed the use of two groups of males and two groups of females. So far as possible care was taken to include at least one rat from each litter in each of our four experimental groups. Each rat was given a number and was further marked for easy identification by the usual method of slitting the ear. The body weight of each animal was accurately determined upon a torsion balance at the start of the experiment and at short intervals thereafter.

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Preparation of diet. Four diets as shown in table 1 were fed. These were "meat diet," "casein diet," "meat and yeast diet," and "casein and yeast diet."

The *meat* used was good "round steak." This was bought in large quantity, the muscle separated and all fat and connective tissue stripped carefully from the lean portion. Much time and care were used in following out the small connective tissue septi, so that the final product was only the lean muscle part. This was twice ground very fine in an electric meat grinder and the resultant mass mixed by hand for one hour to insure a homogeneous product. This ground meat was then dried by spreading it in a thin layer over a broad platter placed over an electric plate set at low heat. More rapid drying was secured by allowing the air current from an electric fan to play upon the thinly spread

TABLE 1

List of diets employed (values in percentages)

CONSTITUENTS	DIETS			
	Casein	Casein-Yeast	Meat	Meat-Yeast
Meat			20	20
Casein	20	20		
Starch	63	63	63	63
Butter fat	15	15	15	15
Salt mixture	2	2	2	2
Yeast (dry)		5 per cent added		5 per cent added
Nitrogen content of diet	2.79	3.17	2.70	3.12

meat layer. When dry enough to break up readily the meat was placed in a small ventilated electric oven and heated over night at 100° to 105°C. It was then ground to a fine powder and again mixed thoroughly. This dried meat powder was then used in making up the "meat diet," and the "meat and yeast diet."

In preparing the *casein* for use in the experiments, commercial casein was soaked in a large volume of water for twelve hours and then dissolved by stirring in dilute sodium hydroxide. The casein was precipitated by adding dilute acetic acid very carefully and with constant stirring until the maximum precipitate was obtained. After settling, the supernatant liquid was siphoned off. This process of solution and precipitation was repeated three times. The final casein precipitate was washed again with water, followed by alcohol and ether in turn. The casein was then dried, ground and mixed as already described for meat,

and the resultant casein powder was used in making up the "casein diet," and "casein and yeast diet."

The *butter fat* used in the diets was obtained by separating the melted fat of *unsalted* butter from the sediment of curd and water. The *salt mixture* (3) was prepared from chemically pure salts and had the composition shown in table 2.

The dry materials for the diet were mixed very thoroughly in a large mixing bowl, and melted butter was added slowly with constant stirring. The food mixture was then sieved to break up any lumps that formed on the addition of the butter-fat. The mixed foods, as well as

TABLE 2

Salt mixture

SALT	AMOUNT
	grams
Calcium carbonate	134.8
Magnesium carbonate	24.2
Sodium carbonate	34.2
Potassium carbonate	141.3
Phosphoric acid	103.2
Hydrochloric acid	53.4
Sulphuric acid	9.2
Citric acid	111.1
Ferric citrate	6.34
Potassium iodide	0.020
Manganese sulphate	0.079
Sodium fluoride	0.248
Potassium aluminium sulphate	0.0245

the individual dietary constituents such as meat and casein powders, were kept in air-tight containers in a freezing tank.

The yeast added to the diet was a per cent quantity by weight of the total diet. For example, 5 per cent of dried yeast by weight was incorporated into the mixture of dry substances (casein or meat, starch and salt-mixture) before the addition of the melted butter.

The diets as fed and their nitrogen contents are given in table 1.

Experiments on group I. This group included nine male rats, no. 1 to 9 inclusive. It was planned to feed these rats for a time on a diet which was inadequate for growth and when the failure to grow at the normal rate had been established to add a little yeast to the diet and see if normal growth followed. The diet fed was the "casein diet"

listed in table 2. This diet included 20 per cent casein, 63 per cent starch, 15 per cent butter and 2 per cent salt mixture (see table 1). This diet is not satisfactory for growth because it lacks "water-soluble B."

The diet as we fed it was really an artificial milk so far as food value was concerned with the exception that the "water-soluble B" of the milk was not present.

TABLE 3

Experiments on group I. Males, cage I, casein

RAT NUMBER	1	2	3	4	5	6	7	8	9
LITTER NUMBER	1	1	2	3	3	3	5	6	6
DATE	WEIGHT								
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21	40	41	58	31	28	29	59	40	35
February 28	43	47	70	31	30	32	73	46	45
March 7	42	47	75	29	30	30	70	*	48
Casein and yeast, March 7, 1918									
March 11	43	53	79	31	35	32	76		52
March 14	45	56	90	37	37	32†	72		53
March 18	46	57	98	41	40	‡	82		52
March 21	49	63	110	45	43		91		52
March 28	49	67	118	47	53		102		§
April 4	70	92	149	68	67		134		
April 11	93	111	170	87	91		159		
April 18	114	130	192	113	113		177		

* Dead, March 6, 1918.

† Sick.

‡ Dead, March 15, 1918.

§ Chloroformed March 28, 1918.

Table 3 contains the observations from the animals in group I. At this stage in the life cycle of the young rat, it should about double its weight in an interval of fourteen days. However, none of these rats in any way approached such gains in body weight. In fact, some of them showed an actual loss and the maximum gain was one from 58 grams to 75 grams shown by rat 5. This was a gain of less than 30 per cent whereas the normal gain should have been about 100 per cent. On the other hand, rat 4 showed an actual drop in body weight from 31 grams

to 29 grams and rat 6 also dropped in body weight from 32 grams to 30 grams in seven days.

After two weeks a little yeast was added to the diet and every rat showed an immediate gain in weight as may be seen by examining table 3. The body weight gains were not very large at first because of the pronounced "nutritional shock" which the eating of the inadequate diet gave the young rats in their very early days. That is, they about

TABLE 4

Experiments on group II. Males, cage II, casein and yeast

RAT NUMBER	10	11	12	13	14	15	16
LITTER NUMBER	1	1	2	3	3	5	6
DATE	WEIGHT						
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21	39	38	57	30	31	57	52
February 28	64	59	83	52	58	89	79
March 7	88	81	120	78	85	114	103
Casein March 7							
March 11	83	75	121	76	86	111	103
March 14	82	74	123	75	85	105	102
March 18	73	63	122	70	83	98	95
March 21	71	63	121	67	80	93	91
March 28	61	54	114	59	72	84	79
Casein and yeast March 28							
April 4	80	68	145	80	96	112	105
April 11	98	81	167	98	125	137	124
April 18	125	114	190	128	157	168	151
April 25	147	128	205	149	162	192	165
May 2	153	142	214	165	172	205	177
May 9	168	152	223	173	181	210	185

doubled their weight in four weeks whereas other rats from the same litters when fed the same diet in their early days more than doubled in weight in two weeks (see table 4). The preliminary "stunting" of the rats by the defective diet wore off in large part at the end of the fourth week of the yeast diet and a rapid growth developed. Rat 3, for example, weighed 75 grams at the start of the yeast diet and six weeks later his body weight had mounted to 192 grams, whereas rat 5,

which weighed only 30 grams at the start of the yeast diet, showed a body weight of 113 grams six weeks later. The growth curve of this rat is reproduced in chart 1.

In our experiments on group I nearly all the rats *maintained* their body weight during the first two weeks of the experiment. Had they been *adult* rats this condition would have been satisfactory. However, they were *very young* rats and needed a diet adequate for growth and such a diet was furnished as soon as we added a little yeast with its water-soluble vitamine.

Experiments on group II. This group included seven male rats, no. 10 to 16 inclusive. The animals were given an adequate diet of casein and yeast as listed in table 1. This diet was satisfactory for growth because of the presence of the yeast and every rat showed immediate and pronounced increases in body weight. In fact, every rat doubled in weight in two weeks and rat 14, in growing from 31 grams to 85 grams in two weeks, *nearly tripled its weight.*

At the end of the fourteen days the yeast was eliminated from the diet and the effect upon the nutrition of the animals was very marked. Not only did the very rapid growth cease but there was *an actual loss in weight.* Thus rat 14 which had gained 54 grams in two weeks while eating a diet containing yeast, showed a *loss of 5 grams* when he was no longer fed yeast. The loss in weight was very nicely shown in the case of rat 11. This rat increased in weight from 38 grams to 81 grams during the two weeks of yeast feeding and decreased to 54 grams at the end of three weeks' feeding on a yeastless diet. The growth curve of this animal is shown in chart 1.

In order to try the influence of yeast at a later stage in the life of the rats, a third period was started at this point and the original diet of casein and yeast was again fed. The same rapid growth was again in evidence throughout an interval of six weeks in spite of the fact that the rats had been "stunted" by eating an inadequate diet for a period of three weeks just previously. In fact, some of the rats showed a weight well above 200 grams at the end of the period.

Experiments on group III. Nine female rats, no. 17 to 25 inclusive, were employed in this group. They were fed the "meat diet" listed in table 1. This diet was the same as the "casein diet "with the exception that round steak, dried and powdered was substituted for the casein.

On this diet the rats failed to show normal growth (4) although their growth was rather better than that shown by rats from the same litters

when fed the "casein diet." Rat 24 is a notable exception (see table 5). This rat weighed 53 grams at the time she was placed on the meat diet and weighed only 55 grams over a month later. Every rat showed initial body weight gains of varying degree but in all instances these initial gains were followed by ultimate losses at the end of about a month. In other words, a diet containing good beef, butter, mineral matter, plenty of energy and an abundance of the important vitamine "fat-soluble A," was not adequate for the growth of the rats.

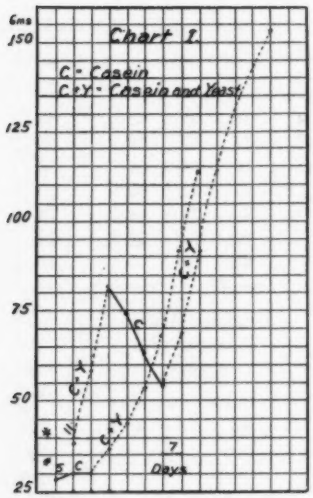

Chart 1

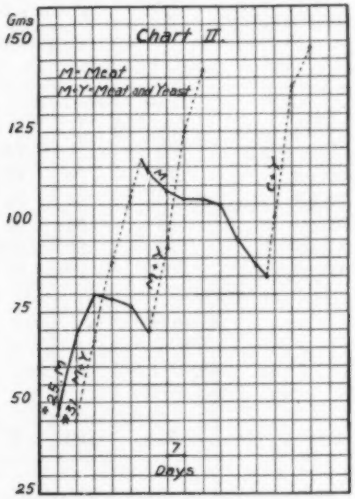

Chart 2

However, the addition of a little yeast made the diet adequate and the rats grew rapidly as shown by the tabulated data in table 5. *Rat 25, for example, lost 8 grams in weight during the last two weeks upon the meat diet and gained 55 grams in the two weeks immediately following simply through the addition of a little yeast.* (*See chart 2.*) Other gains almost as spectacular are recorded in table 5, all of which further emphasize the fact that yeast possesses unusual power to promote growth.

The case of rat 24 is worthy of special note. While on the diet of meat and yeast this rat contracted pneumonia. She was isolated weighing 105 grams and two weeks later in spite of her condition she weighed 130 grams.

Experiments on group IV. Seven female rats, no. 26 to 32, were used in these experiments. They were first fed a diet of meat and yeast (see table 1). On this diet they showed very satisfactory growth as will be seen by examining the data contained in table 6.

TABLE 5

Experiments on group III. Females, cage III, meat

RAT NUMBER.....	17	18	19	20	21	22	23	24	25
LITTER NUMBER..	1	2	2	3	3	4	4	5	6
DATE	WEIGHT								
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21..	38	54	51	31	31	36	42	53	46
February 28..	60	77	74	47	45	54	62	76	68
March 7.....	72	86	85	51	45	69	72	65	80
March 14.....	75	83	89	49	35*	67	71	62	79
March 18.....	75	86	90	48	†	65	71	61	78
March 21.....	72	86	96	48		65	70	58	78
March 25.....	69	92	99	49		63	65	55	78
	Changed March 25, meat plus yeast					Changed March 25, meat plus yeast			
April 1.....	85	90	97	37*		84	74	65	70
		Changed April 1, meat plus yeast							Changed April 1, meat plus yeast
April 8.....	92	110	113			105	95	92	93
April 15.....	100	124	131			130	119	105*	125
April 22.....	102	127	132			130	127		142
April 29.....	105	134	138			132	132	130	147

* Sick.

† Dead March 15, 1918.

When the yeast was no longer fed, the rats lost weight as in the previous tests. For example, rat 31 grew from a weight of 45 grams to one of 117 grams between February 21 and March 18 when fed a yeast diet, whereas her weight decreased from 117 grams to 85 grams between

March 18 and May 6, when fed *a yeastless* diet. Chart 2 shows the growth curve of this rat.

TABLE 6

Experiments on group IV. Females, cage IV, meat plus yeast

RAT NUMBER	26	27	28	29	30	31	32
LITTER NUMBER	1	2	2	3	3	4	5
DATE	WEIGHT						
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21	36	55	54	32	29	45	51
February 28	57	82	82	54	51	66	82
March 7	40	108	100	72	67	89	100
March 14	*	122	109	65§	68	106	114
March 18	†	126	124		73	117	126
March 21				66‡			
•	Meat March 18						•
March 21		128	126		75§	113	126
March 28		128	115		63§	108	103§
April 4		127	117		76§	106	
April 11		130	114			106	
April 18		129	109		94	105	
April 25		130	102		90	95	
May 2		134	102		89	88	
May 6		128	97		89	85	
	Casein and yeast May 6						
May 9		146	113		98	101	
May 13		157	126		108	120	
May 16		159	137		117	137	
May 23		166	145		130	148	

* Dead March 8, 1918.
† Dysentery.
‡ Dead.
§ Sick.

A third period of seventeen days was used in this experiment. During this interval each rat was fed the usual casein-yeast diet and the customary gains in body weight were observed.

CONCLUSIONS

The addition of compressed yeast to a diet lacking the water-soluble vitamine causes an immediate and pronounced increase in body weight. This increase is more rapid and pronounced when the diet contains casein than when the protein of the diet is furnished by lean meat.

Compressed yeast may be heated to 105°C. without losing its growth-promoting properties.

The authors are under obligation to Dr. Clarence A. Smith for assistance rendered.

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ALTERATIONS IN THE ACTIVITY OF THE TERRAPIN'S HEART RELATIVE TO SLIGHT CHANGES IN THE P_H VALUE OF THE PERFUSATE

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The influence upon the beating heart of a change in the reaction of the perfusate has been studied by a number of investigators. Thus Gaskell (1) reported in 1880 that perfusion with dilute alkali caused the heart to stop in systole, while the effect of a weak acid was to stop it in diastole. Mines (2) reviewed the effect of the hydrogen ion concentration and made a study of its action upon the various phases of the heart beat. Jerusalem and Starling (3), working upon the isolated heart of the frog, found that CO_2 in an excess of oxygen had an injurious effect, causing a relaxation which definitely diminished the ventricular output. These authors believed, however, that a certain tension of CO_2 in the blood was beneficial to activity. Ketcham, King and Hooker (4) found that CO_2, 2.5 per cent in an excess of oxygen, depressed the activity of the isolated terrapin's heart and that 3 per cent of the same had a similar effect upon the isolated mammalian heart. Mines (5) attempted to reconcile the apparently divergent results of Ketcham, King and Hooker, and Jerusalem and Starling upon the supposition that the latter had started with a perfusate which was too alkaline and the beneficial action reported by them he ascribed to the action of the CO_2 in slightly increasing the P_H[1] value to a level nearer the optimum. Mines showed that a heart perfused with a Ringer mixture of $P_H = 10.2$ was greatly benefited by the addition of sufficient CO_2 to raise the P_H to 6.9. However, if "neutral Ringer" were used to start with, any addition of CO_2 caused a depression of activity.

Clark (6) found that when CO_2 was bubbled through Ringer in a quantity sufficient to raise the P_H from 8.3 to 6.7, a markedly beneficial re-

[1] It may be pointed out that P_H is simply the cologarithm, i.e., the logarithm of the reciprocal of the C_H. Thus $C_H\ 0.35 \times 10^{-7} = P_H\ 7.45$.

sult was often obtained upon perfusion. This he laid to a specific action of the CO_2 upon the heart and not to any increase in the P_H of the solution.

Two features of the work thus far published upon the influence of a change of the hydrogen ion concentration upon the heart are to be noted. In the first place those investigators who have expressed the characteristics of their perfusates in percentage CO_2 have not stated the corresponding P_H values. Clark points out that Ringer's mixture containing $NaHCO_3$ 0.01 per cent and NaH_2PO_4 0.001 per cent has a P_H value of 8.3, and that addition of 1 per cent CO_2 will raise the P_H to 7.0, while the further addition of 1 per cent raises it to 6.6. So that the workers who have not measured the actual change in the P_H value have probably dealt with acid solutions and the deleterious results observed were due to the acidity. In the second place, those who have measured the P_H value of their solutions have worked with differences in P_H value far greater than any which could take place in the blood even in cases of severe acidosis.

It has seemed desirable, therefore, to make a study of the effect of variations of the P_H of the perfusate within substantially normal limits. To this end a series of experiments was carried out on the heart of the terrapin.

EXPERIMENTAL

The heart was disclosed after removal of the plastron and a cannula inserted in a vena cava. A second cannula for outflow was inserted in the aorta. The perfusion pressure was regulated by Marriott bottles and manometer or by means of a chimney cannula after Mines. In all cases the pressure was kept as nearly constant as possible especially during the change from one perfusate to another and at a level just sufficient to cause dilatation of the auricles in diastole and yet permit contraction in systole.

Record was made of the beat of both auricles and the effect of tone changes in one chamber upon the record of another was as far as possible avoided. One side of the ventricle was anchored and the beat recorded by means of a thread from the other side. The auricular beats were recorded by means of a thread led off from the tip of each chamber. By means of pulleys the three chambers were made to record their beats simultaneously upon a smoked surface.

Ringer's solution was made up in quantities of six to twelve liters according to the following formula:

NaCl	0.7	$NaHCO_3$	0.02
KCl	0.25	NaH_2PO_4	0.001
$CaCl_2$	0.20	Water to	100

Such a mixture was found to have a P_H from 7.8 to 8.0. The desired P_H was then obtained by removing a portion to a smaller bottle and adding M/10 HCl or M/5 $NaHCO_3$. The P_H was measured by adding five drops of a 0.01 per cent solution of phenolsulphonephthalein to 3 cc. of the Ringer's and comparing with standard tubes. Within the range used in these experiments a change of P_H of 0.1 could be easily detected.

The P_H of the blood of the terrapin used, determined by the method of Levy, Rowntree and Marriott (7), was found to be 7.4 to 7.5. This was taken as the normal for the perfusates and variations were made to both sides. In no case, however, was a P_H of less than 8.0 or more than 7.1 employed. The majority of the experiments involved changes from 7.3 to 7.6.

The results of a series of experiments are shown in the accompanying charts and figures.

The record in figure 1 shows, in general, the results of such changes in the P_H of the perfusate. The upper record registers the beat of the left auricle, the middle that of the right and the lower that of the ventricle. A solution of P_H 7.4—approximately that of the blood—was passed through the heart for a half-hour and was then replaced by one of P_H 7.3. There followed at once an increase in the amplitude of the beat in all chambers. This was most striking in the right auricle where the height of the record of beat increased from 13 to 18 mm. in ten minutes. In the ventricle the increase was not so great but was easily perceptible.

At the arrow a perfusate of P_H 7.5 was introduced with very characteristic results,—a falling off in the amplitude of beat of all chambers and a gradual increase in tonus. Further decrease in P_H, to 7.7 and 7.8 as indicated upon the record, served to accentuate these results until in the ventricle the amplitude of contraction had been reduced by a half and there was a perceptible increase in tonus. The auricles soon decreased in size so that their beats could no longer be recorded.

When this condition had been reached the P_H was raised to 7.3. The tonus, both in the auricles and ventricle, was at once lowered and the amplitude slowly increased until, fifteen minutes later, the beats were again strong and regular. At the arrow Ringer of the same P_H as the blood was again introduced with a slight decrease in the amplitude in all chambers.

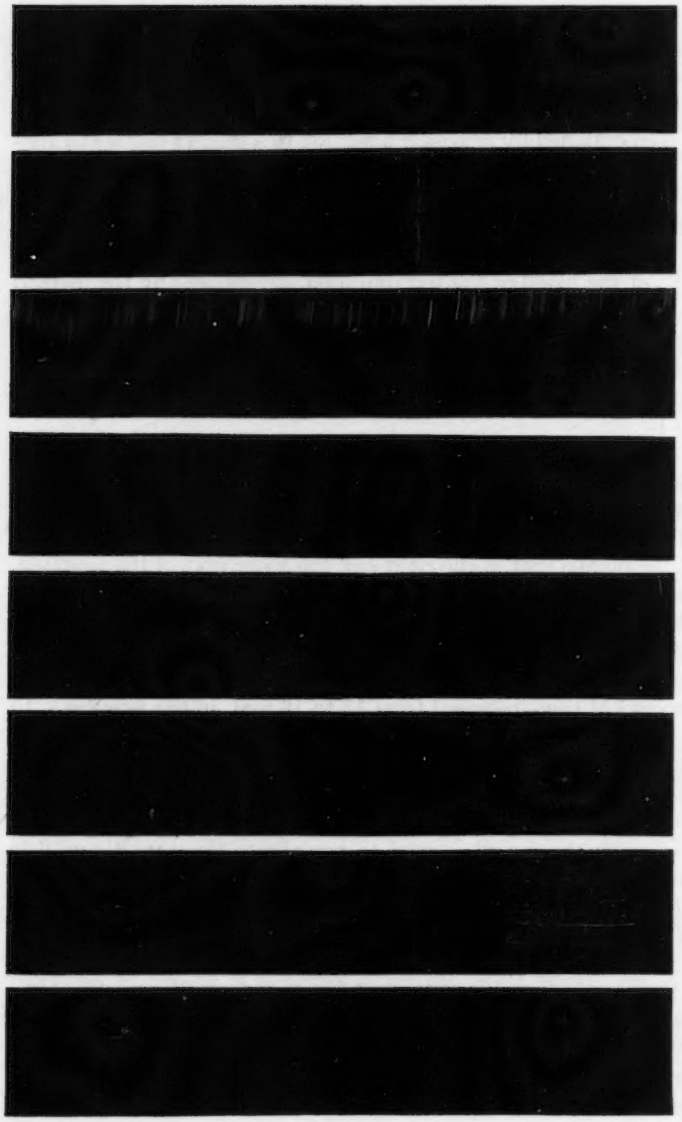

A 1:10
P_H 7.4
B 1:40
P_H 7.3
C 1:50
P_H 7.5
D 2:10
P_H 7.7
E 2:30
P_H 7.8
F 2:45
P_H 7.3
G 2:55
P_H 7.3
H 3:10
P_H 7.4

Fig. 1

In the experiment from which figure 2 was taken a series of relatively rapid changes of P_H was made. Perfusion commenced at a P_H of 7.4 and after ten minutes this was lowered to 7.6 for twenty minutes. This caused an increase in tonus and a decrease in amplitude as usual. Change to P_H 7.3 for a half-hour produced some relaxation but not sufficient to bring the tonus back to the normal level. Upon lowering to P_H 7.4 for ten minutes there was some rise in tonus. P_H 7.6 for ten minutes caused a sudden large rise which however fell off sharply when a perfusate of P_H 7.4 was introduced. After ten minutes of this a P_H of 7.3 caused some further relaxation. A change to P_H 7.5 caused a slow but very definite increase in tonus which was then decreased by a P_H of 7.4.

Here the increase in tonus in the auricles produced by the more alkaline perfusates was not entirely balanced by the action of those slightly more acid than the blood so that the tonus level at the end of the experiment was somewhat higher than at the start. As in the former experiments, the changes produced in the ventricle were smaller and somewhat more sluggish than those in the auricles.

These results point clearly to the action of slight alterations of P_H of the fluid bathing the heart upon two important phases of its activity, namely, tonus and amplitude. There is invariably a rise in tonus following a decrease in the H-ion content of the perfusate, while a slight increase in acidity causes a corresponding fall in the tonus level. There is an accompanying effect upon the amplitude of contraction though by no means as regular nor as marked as that upon the tonus. But in general there is an increase in amplitude with the more acid perfusates and a decrease with the more alkaline. This result may be due not entirely to the direct effect of the P_H upon the contractile tissue but partly to intra-auricular and intra-ventricular tension. Rate was not perceptibly affected.

It should be noted that in no case was the perfusate made actually acid—a P_H of less than 7.0—but merely somewhat more acid than the blood. The injurious effects of an acid perfusate as observed by Mines (2) were therefore not encountered.

Gaskell (8) has shown that when an alkaline perfusate is passed through the blood vessels there is a decrease in outflow due to an increase in the vascular tone. When an acid solution is used the opposite occurs. Since the heart has embryologically the same muscle layers as the blood vessels and is formed from a single vessel, it is not unreasonable to suppose that it might be similarly affected. And, indeed, this has been shown to be the case.

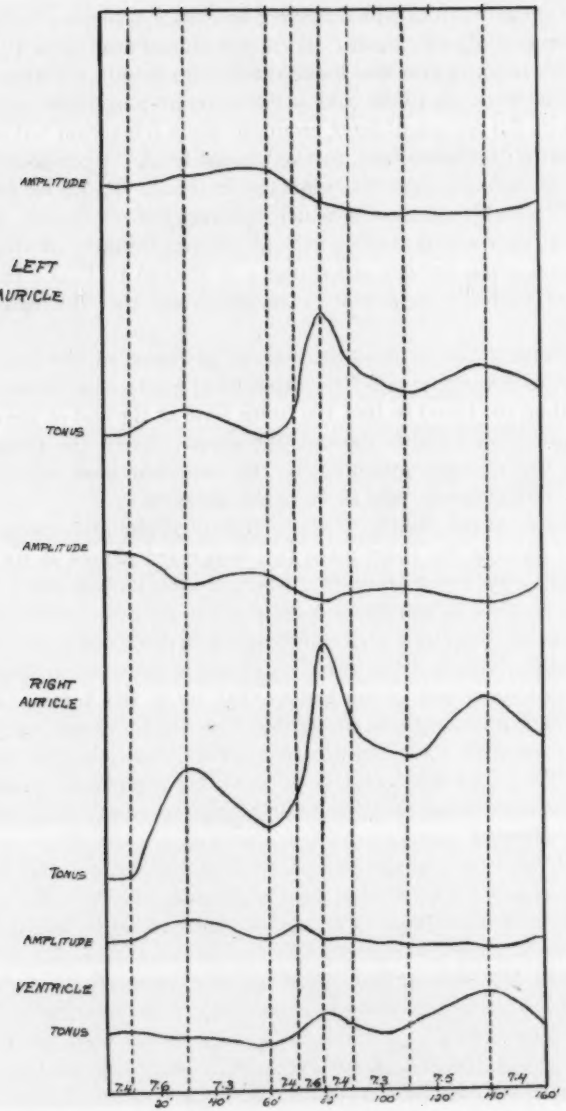

AMPLITUDE
LEFT AURICLE
TONUS
AMPLITUDE
RIGHT AURICLE
TONUS
AMPLITUDE
VENTRICLE
TONUS
7.4
7.6
20'
7.3
40'
60'
7.4
7.6
80'
7.4
7.3
100'
7.5
120'
7.4
140'
160'

Fig 2

Gesell (9) has made a most careful study of the effect of tone changes upon the cardiac output and finds that perfusion of the terrapin's heart with a constant venous pressure shows variations in ventricular output resulting from changes in auricular tone. This he attributes to actual changes in the auricular output, which cause greater length and tension of the ventricular fibers, thereby producing greater efficiency of ventricular systole. He points out that as auricular tone is reduced the output per beat increases. To quote,

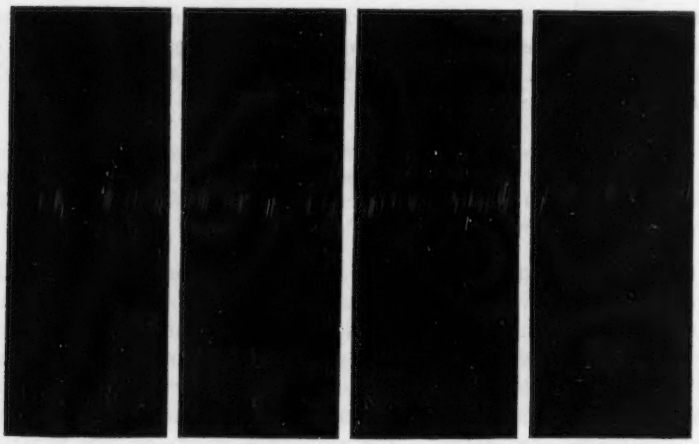
> The long muscle is more efficient than the short. The volume of the auricle increases as the cube, its surface as the square of the radius. Therefore, the greater the volume of the auricle the greater will be the volume change per unit length of shortening of auricular fiber.

Hence in the experiments above, regardless of any increase in the amplitude of contraction, the volume output of the auricles was increased with the more acid solutions. As Gesell has also pointed out, such an increase would produce a greater length and tension of ventricular fiber and hence a more efficient ventricular systole. In some instances this would amount to an actual increase in the amplitude of the ventricular contraction. The amplitude effects on the ventricle in some of the experiments mentioned above may be explained in this way.

But there was a very definite tonus effect in the ventricle entirely independent of the filling action of the auricular systole. In the experiment, the record of which is shown in figure 3, A and B, there was a condition of reversed sequence in which the ventricle led, the sequence being ventricle-right auricle-left auricle, and both auricular systoles occurred during the contraction phase of the ventricular beat (see fig. 3 A). The effect of the auricular output upon the length and tension of ventricular fiber was thus entirely eliminated. But substantially the same results as regards tonus were observed as in the cases where the hearts were beating in normal sequence, i.e., an increase in tone with greater alkalinity and a loss with a decrease in alkalinity below that of the blood. The ventricular tonus may be affected, therefore, by alterations of the P_H of the perfusate independently of the effect of such changes upon the auricular output.

The effects of change of P_H upon the amplitude of contraction of the ventricle in the case of reversed sequence were not so marked as in hearts beating with normal rhythm. This would seem to support the

idea again that an increase in ventricular amplitude observed, may be due largely to the increased intra-ventricular tension produced by an increase in auricular output.



A Showing reversed rythm | B 2:10 P_H 7.3 | C 2:40 P_H 7.6 | D 3:00 P_H 7.4

Fig. 3

DISCUSSION

The results of the present study, as presented above, would indicate that the heart of the terrapin is sensitive to relatively slight changes of H-ion concentration in the surrounding fluid. A decrease in acidity causes a prompt rise in tonus which is more evident in the auricles but is also present in the ventricle, and is accompanied by a falling off of the amplitude. An increase in acidity to a point slightly less alkaline than the blood produces a loss of tonus and an increase in amplitude of contraction in all chambers.

While the effect of auricular tone upon cardiac efficiency has been made clear through the work of Gesell, the question of the cause and control of such tone has been the subject of considerable controversy. Fano (11) reported in 1887 that strips of the turtle's auricle normally showed rhythmic tone changes. Botazzi (12) separates the movements of the auricular muscle of the turtle into two kinds and ascribes their independence to the structures of the myocardiac cells. To the sar-

coplasm he attributes the function, in its tonic contraction, of supporting the anisotropic substance in its quicker contractions.

Botazzi and Grünbaum (13) point to the similarity existing between auricular tone changes and the contractions of smooth muscle. They contend, nevertheless, that tonus must be a function of the sarcoplasm and that the rapid clonic contractions of the auricular muscle which are superimposed upon the slower tone changes are due to the anisotropic substance of the fibrillae. (Smooth muscle, in their opinion, plays no important rôle in the rhythmic phenomena).

Rosenzweig (14) reports a layer of smooth muscle directly beneath the auricular endothelium in the turtle, which he regards as a continuation of the tunica media of the great veins, becoming thinner with distance from the mouth of the veins until only isolated fibers of smooth muscle are demonstrable in the ventricle. He attributes the tonic contractions of the auricles to this layer and the more rapid contractions to the cardiac muscle proper.

Botazzi (15), (16) observed that vagus stimulation produced rhythmic oscillations of tone in tone-free auricles. The vagus, therefore, was regarded as positively tonotropic. The sympathetic on the other hand caused oscillations to cease and generally reduced the tonus level. This nerve therefore was regarded as negatively tonotropic. Ouinuma (17) confirmed these observations.

The results of the work presented above seem to add one factor to the discussion. It is clearly shown in the preceding paragraphs that the ventricle exhibits changes in tone in response to chemical stimulation quite apart from any effect of the auricular beat upon the filling of the ventricle. With the data in hand it is quite impossible for us to account satisfactorily for this result either upon the sarcoplasm theory of Botazzi or upon the basis of the presence in the ventricle of isolated fibers of smooth muscle as reported by Rosenzweig.

CONCLUSIONS

1. Slight alterations in the P_H value of the perfusate within substantially normal limits, and in all cases upon the alkaline side of neutrality cause definite changes in the activity of the terrapin's heart.

2. A decrease in acidity to a P_H value of 7.6 to 7.8 causes marked changes in two phases of activity. The tonus especially in the auricles rises rapidly and the amplitude is decreased.

3. A slight raising of the P_H value to 7.3 increases the cardiac output in either or both of two ways, i.e., by a decrease in tone and hence an increase in initial volume, or by an increase in the amplitude of beat.

An experiment is reported showing that, in the case of a reversed sequence, tonus changes can be produced in the ventricle quite independently of any changes in the filling action of the auricular beat.

I wish to express my thanks to Dr. C. G. Rogers of Oberlin College, under whom the study was begun, and to Dr. Charles D. Snyder of the Department of Physiology of Johns Hopkins Medical School, for his kindness in allowing me space in his laboratory and for many helpful suggestions.

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A COMPARATIVE STUDY OF CARBON DIOXIDE PRODUCTION DURING STARVATION IN PLANARIA

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Earlier studies of the physiological condition of *Planaria dorotocephala* by the susceptibility method led to the conclusion that during starvation the rate of oxidation actually increases, so that an animal reduced from larger to smaller size by starvation is in almost the same metabolic condition as indicated by susceptibility to lethal concentrations of various agents, as a young fed animal of the same size (1), (4), (6, chap. vii). Between such animals, however, one difference was found to exist: the well-fed animal possesses a very much greater capacity than the starved animal for acclimation to various inhibiting agents in low concentrations and to other injurious conditions. When the starved animal is fed these differences disappear and its physiological condition is indistinguishable, so far as concerns susceptibility, from that of the well-fed, growing animal of about the same size. Even a single feeding after a period of starvation is sufficient to increase to a considerable extent the capacity for acclimation. If susceptibility relations are in any degree a measure of physiological condition, a period of starvation with the accompanying reduction in size, followed by resumption of feeding, brings about a process of rejuvenescence.

In connection with the earlier work a few estimations of carbon dioxide production were made by means of the Tashiro biometer, animals reduced by several months of starvation being compared with well-fed animals of the same size (4, p. 434). These estimations indicated that the CO_2 production of the starved, reduced animal was about equal to that of the well-fed, growing animal of the same size and much greater than that at the beginning of starvation. The biometer data agree in general with the susceptibility data but of course give no clue to the nature of the changes concerned in the increase of acclimation capacity on resumption of feeding.

During the last two years the earlier work has been supplemented by comparative colorimetric estimations of CO_2 production, by further work with the biometer, by determination of susceptibility to lack of oxygen in starved and fed animals and by repetition and extension of the experiments on susceptibility to chemical agents. The data on CO_2 production are presented below.

METHODS

The experiments are directly comparative in all cases: equal weights of the animals to be compared are placed in equal volumes of water containing the indicator in sealed tubes, the change of color with increasing acidity serving as a measure of CO_2 production.

Phenolsulphonephthalein, the indicator found to be most satisfactory, is used in a concentration of about 1/150,000 in water from the same source as that in which the stocks of worms are kept. Although the object of the experiments was the determination of the differences in CO_2 production rather than the actual amount of CO_2 produced, the experimental tubes were compared with the standard tubes prepared by Hynson, Westcott & Dunning with indicator in buffer solutions of different known pH, and the pH recorded.

That the increase in hydrogen ion concentration in the water in which the worms are kept is due to CO_2 and not to other acids is indicated by the fact that, on standing or shaking with air after removal of the worms, the original color of the indicator returns. The substance produced by the worms, which increased the hydrogen ion concentration, is therefore removed by this procedure and the probability that the animals secrete any other substance than CO_2 which acts as an acid in water and can be removed in this way is negligible, and other experiments show that they do produce considerable quantities of CO_2. The concentration of the indicator is the same in experimental and in buffer solutions. In this concentration, 1/150,000, and in fact in much higher concentrations this indicator is not appreciably toxic to planarians, and even in 1/10,000 the animals live indefinitely, though it acts as a weak inhibiting agent. The solutions of the buffer series differ by 0.1 pH, or at the ends of the series by 0.2 pH, but since color differences corresponding to far less than 0.1 pH are readily distinguishable with a little practice over those portions of the range where the indicator is most sensitive, the second decimal place is determined by estimation, or in some cases the sign + or − is added to the pH number of the color nearest the experimental color.

The well water used for experiment has a pH of 7.4 to 7.6 when first pumped, but on standing for some time or after five to ten minutes shaking with air pH = 7.6 to 7.8 or sometimes even 8.0. Since it seemed desirable in many cases to vary the pH of the starting point, the water was sometimes used at pH 7.6 to 7.7, while in other cases it was brought to 8.0 or 8.1 by shaking with air or by adding if necessary a few drops of sodium carbonate m/100.

The worms are weighed "dry", that is, with no excess of water. The procedure found most satisfactory is as follows: the worms to be weighed are dropped on filter paper from a pipette with as little water as possible and after about thirty seconds each animal is transferred to the container on the balance pan on the tip of the blade of a very narrow-bladed lancet which has been slightly vaselined to prevent as far as possible the adhesion of water. The container is a small glass vessel, e.g., the bottom cut from a small vial, and the worms are transferred to it by drawing the lancet blade carefully across its edge.

The container is of course balanced before the worms are added and the transfer and weighing of the worms must be done as rapidly as possible to avoid injury from drying, but since the total weight of even fifteen or twenty worms reduced by starvation is only a few milligrams, it must be reasonably accurate. After some practice it is possible to estimate rather closely the weight of a particular lot of worms and to reduce the time necessary for weighing by arranging the weights accordingly before the worms are transferred. Two minutes exposure to air which is not extremely dry will not usually injure the animals, but this is near the limit. As soon as weighed the worms are returned to water.

After approximately equal weights of the worms to be compared have been obtained, each lot is placed in a clean pyrex tube, rinsed twice in the indicator solution which is to be used, the tube is then filled with the solution and sealed.

Two methods of sealing have been used: first, that described by Haas (7), in which a tightly fitting paraffined rubber tube several centimeters long, attached to the open end of the pyrex tube, is closed by a clamp, after filling with the indicator solution to a level above the clamp to avoid air bubbles. This method has some disadvantages for motile animals such as Planaria, for some individuals may creep into the rubber tube, where they cannot be seen, and it is desirable to keep all in sight at all times in order to make certain that all remain in good condition and to determine whether unusual motor activity, which of course increases CO_2 production, occurs. Another method of sealing which has proved satisfactory consists in closing the tube first with a very

tight plug of acid-free absorbent cotton soaked in the indicator solution, care being taken to exclude bubbles. This plug is pushed far enough into the tube to leave room for at least 1 cc. of solution above it and for a paraffine seal. A small bubble of air is left between the paraffine and the upper solution to prevent leakage from changes in pressure in consequence of slight temperature changes. Diffusion through the cotton plug is so slow that it is of no significance during the period of the experiment.

After the tubes are sealed they are of course kept under as nearly as possible the same conditions and from time to time the different lots of a series are compared directly with each other and with the standard tubes. The extreme range of color change used is from pH 7.6 to 8.1 at the beginning to pH 7.0 at the end, but in most cases only about half this range is used.

In order to avoid as far as possible errors resulting from differences in motor activity of the different lots, the heads are removed in almost all experiments one to twenty-four hours before the pH determinations. It has been found that except as regards motor activity, the removal of the head has very little or no appreciable effect on total CO_2 production. The headless animals are much less active than the normal, though of course able to react in the normal way when sufficiently stimulated. If the tubes are carefully handled and kept from sudden and great changes in light intensity, the headless animals usually remain motionless throughout the period of the experiment after the first few minutes.

Carbon dioxide production in relation to age. Since the present paper is a contribution to the question of the effect of starvation on the physiological age of the animals, it is necessary to present first some data concerning CO_2 production in well-fed animals of different physiological age, size being taken as a criterion of age in worms of a given stock (6, chap. iv). The following table gives a characteristic series.

TABLE 1

Series 666. Lot I, old animals. Lot II, young animals. Both well fed. In both heads removed

	LENGTH	NUMBER OF WORMS	WEIGHT	TIMES IN HOURS AND pH				
				0	2	3	5	6½
	mm.		*grams*					
I	25	3	0.0481	7.7	7.45	7.3+	7.25	7.2+
II	7	36	0.0470	7.7	7.3+	7.2	7.1+	7.05

Even though the thirty-six young weigh slightly less than the three old animals, they produce CO_2 much more rapidly. Similar comparisons of old and young animals have been repeated many times with worms of different sizes, with and without heads and by different persons, the experiment having been used as a laboratory experiment for students. The result is always the same, a higher CO_2 production per unit of weight in the young than in the old.

In *Planaria dorotocephala* the young worms of a stock are in all probability agamically produced, since gametic reproduction has never been observed in the locality from which the animals are collected (6, p. 386). In other species, however, the differences btween sexually produced young and old are like those between young and old of agamic origin and of the same sort as in *P. dorotocephala*.

Starvation stocks. It has long been known that Plan ria will live for several months without food and during this period will undergo reduction to a small fraction of its original size. The reduction in size is due to the fact that the animal is able to live upon its own structural substance after using up whatever nutritive reserves may be present. The alimentary tract is apparently the chief source of nutrition and undergoes reduction more rapidly than other organs of the asexual animals, but all parts of course undergo reduction more or less rapidly.

In making up starvation stocks careful standardization as regards several factors is necessary. In the first place, since differences in size, in nature or in a general laboratory stock represent differences in physiological age with corresponding differences in CO_2 production, animals of as nearly as possible the same size must be selected for a starvation stock. Second, these animals must be as nearly as possible in the same nutritive condition, for CO_2 production varies greatly with nutritive condition. Third, the larger animals consist physiologically of more than one zoöid (2), (3), (6, chap. vi) and almost invariably undergo fission during the first few days of starvation at a level slightly posterior to the mouth (*aa*, fig. 1). The anterior and posterior products of fission undergo regulation into normal animals (fig. 2, anterior; fig. 3, posterior) and these two animals resulting from fission, the "anterior" and "posterior" animals as they may for convenience be called, show characteristic differences in body-form, rate of reduction and physiological condition during starvation, and the posterior animal often undergoes a second fission and regulation.

These differences between anterior and posterior animals must be briefly considered. The process of regulation in the anterior product of

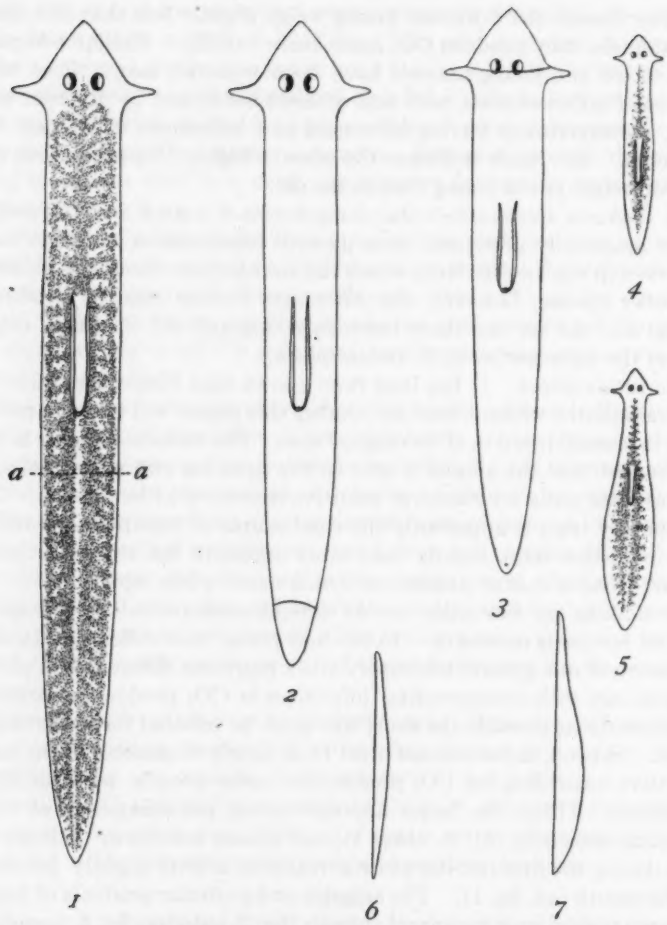

Fig. 1. A large, well-fed individual of *Planaria dorotocephala*, showing alimentary tract: *aa*, level at or near which fission usually occurs.

Fig. 2. Anterior animal resulting from fission

Fig. 3. Posterior animal resulting from fission.

Fig. 4. Anterior animal reduced by about three months of starvation, showing body-form and extent of alimentary tract.

Fig. 5. Posterior animal reduced by about three months of starvation, showing body-form and extent of alimentary tract.

Fig. 6. Outline of starved anterior animal in side-view.

Fig. 7. Outline of starved posterior animal in side-view.

fission consists chiefly in the development and compensatory growth of a new postpharyngeal region (fig. 2), while in the posterior product it consists in the development and growth of a new head, prepharyngeal and pharyngeal region (fig. 3). The anterior animal is physiologically a single individual, except the extreme posterior end, where a second zoöid is usually physiologically distinguishable. The postpharyngeal region of a large worm like that in figure 1 always consists of two or more zoöids (2), (3), (6, chap. vi), and the posterior animal resulting from the fission of such a worm therefore consists of at least two zoöids of nearly equal length. In the anterior animal then practically the whole body is under the control of the dominant head and behaves as a well-coördinated whole, while the two zoöids composing the posterior animal show a certain degree of independence, which becomes more and more evident as starvation progresses.

The alimentary tract of the large, well-fed animal fills most of the body (fig. 1) and since it is not only a digestive organ but the chief place of storage of nutritive reserves, its cells are large and loaded with fat and other substances. Moreover, the bulk of the alimentary tract is greater in the postpharyngeal than in the prepharyngeal region, as figure 1 shows. In relation to its size, then, the posterior animal has a larger volume of nutritive reserves and entodermal tissue as a source of nutrition during starvation than the anterior animal.

These two differences between anterior and posterior animals arising by fission at the beginning of starvation, viz., the less complete coördination and the greater volume of the alimentary tract in the posterior than in the anterior animal, determine certain differences in the course and rate of reduction during starvation. After some three months of starvation (more or less according to temperature) the anterior animal presents the appearance of figure 4, the posterior animal that of figure 5. In the former the head is relatively large, the body slender and of almost uniform width throughout and the alimentary tract has undergone great reduction, all the more lateral portions having completely disappeared and the anterior and posterior terminal portions being reduced in length and diameter. In such animals the spaces in the parenchyma formerly occupied by parts of the alimentary tract are clearly visible in the lateral and terminal body-regions when the worms are brought under slight pressure. In the posterior animal the head is relatively small, the prepharyngeal region slender and the postpharyngeal region much broader, and the alimentary tract is much more reduced in the prepharyngeal than in the postpharyngeal region. Fig-

ures 6 and 7 are outlines of the two animals in side-view to show the differences in the dorso-ventral direction. The anterior animal (fig. 6) is thin throughout, the thickness decreasing in both directions from the pharynx, while in the posterior animal (fig. 7) the anterior zoöid is thin but the posterior zoöid is much thicker. Moreover in these advanced stages of starvation it can be clearly seen that in the anterior animal the whole body is well coördinated while the broad, thick posterior zoöid of the posterior animal is dragged about by the anterior zoöid as a more or less passive mass, although with strong stimulation it may be brought under control.

In the anterior animal starvation and reduction have evidently progressed more rapidly than in the posterior animal as a whole, although in the latter the anterior portion, i.e., the anterior zoöid, being the more active, has undergone reduction to a greater extent than the posterior. The total bulk of alimentary tract in the posterior animal (fig. 5) is much greater than in the anterior animal (fig. 4). If the rate of CO_2 production changes during starvation, we should expect to find the change occurring more rapidly in the anterior than in the posterior animal, and this is actually the case, as will appear below.

In most cases, however, the posterior animal itself undergoes fission and regulation during the first few weeks of starvation, giving rise to two individuals, "anterior-of-posterior" and "posterior-of-posterior," which show essentially the same differences as the anterior and posterior products of the first fission but are of course smaller. Much less frequently anterior animals undergo fission during starvation and in this case the two products, "anterior-of-anterior" and "posterior-of-anterior," are much alike in size and shape except that the regenerated head of the posterior-of-anterior animal always remains smaller than the head of the anterior-of-anterior animal, which is the original head.

Such products of second fission use their body-substance not only in functional activity and partial maintenance of certain organs, but also in regulation and growth of new parts, consequently a second fission during starvation must accelerate the progress of starvation.

In addition to the differences in size due to fission, differences in size which may be called accidental appear in starvation stocks. Of two animals of the same size at the beginning of starvation one may contain a larger amount of nutritive reserves and therefore undergo reduction more slowly than another. Sometimes also one animal eats another and thus obtains sufficient food to delay starvation and reduction to a considerable degree. This cannibalism occurs chiefly where animals

of very different size are kept together in the more advanced stages of starvation, but injured animals and those with dying and disintegrating parts are often attacked and eaten. It is prevented to a large extent by avoiding over-crowding, by isolating animals of different sizes and by removing dying or injured animals as far as possible.

In order to determine what changes occur during starvation, it is necessary not only to standardize the stock at the beginning as regards size, nutritive condition and preceding fissions, but also to distinguish between the different products of first and second fissions or, where this is not possible with certainty, as is often the case in the two products of fission of anterior animals during starvation, to use size as a criterion of stage of starvation. If anterior and posterior, or anterior of posterior and posterior of posterior, or animals of very different size are mixed in the lots used for comparison, the results may be entirely misleading. Since starvation proceeds more rapidly at higher than at lower temperature, stocks to be compared must be kept at the same temperatures.

In all the following experiments all these points have received attention. All starvation stocks were made by selecting animals of the same size from one general stock which has been fed in the laboratory three times a week with beef liver during at least two months. Some stocks were composed of anterior or of posterior animals from fissions which had occurred within a few days preceding selection. In others animals like that of figure 1, 25 mm. in length, were selected and either allowed to undergo fission during the first few days of starvation as almost every individual does, or each animal was cut approximately at the fission plane (*aa*, fig. 1) and the anterior and posterior animals resulting were used as starvation stocks. In short, each lot of animals in each experiment is as nearly uniform in all these respects as it was possible to make it.

For long periods of starvation, large physiologically old animals were used, since they undergo more extreme reduction before becoming too small to handle readily, but it has been found that small, young animals reach essentially the same physiological condition after a short starvation period as large, old animals, after a much longer starvation period. In order that animals in different stages of starvation may be available for comparison, starvation stocks are made up at intervals of a few days or a few weeks and kept as nearly as possible under identical conditions.

The earlier stages of starvation. The stocks used for study of the earlier stages of starvation consisted of anterior animals 12 to 14 mm. in length, the starvation period beginning some ten days after fission.

In such animals the regeneration at the posterior end is about completed but the posterior zoöid is not far enough developed for the occurrence of another fission during the first weeks of starvation. The data for five characteristic series are tabulated in table 2.

Table 2 shows the effect of a few days of starvation upon the CO_2 production. In each series animals fed within one or two days (I) are compared with animals starved from three to twelve days (II, III). In the first three series of the table heads were removed before comparison, in the other two the animals were intact. The fourth column, "No. of worms," shows that the starved worms have lost somewhat in weight: in series 656 II, the number $5\frac{1}{3}$ in this column means that the anterior third of a worm was added to the five whole worms to make up the weight. In the columns under "times of record and pH" the pH as observed at different times is given. Records are given only up to seven and one-half hours, but most of the series were continued several hours more, usually at least ten hours in all and in some series eighteen hours. The later readings of pH, however, do not in any case alter the result except that the differences between the two lots compared increase as time goes on.

Although in every series the weight of the starved worms is greater than that of the fed worms, the starved worms always produce less CO_2 than the fed worms. Moreover, in series 643, lot III, with seven days of starvation produces less CO_2 than lot II with three days of starvation. These experiments with short periods of starvation have been repeated frequently and have been used as class experiments with the same result in all cases. There can be no doubt that the total CO_2 production of these animals decreases rapidly during the first few days of starvation.

The later stages of starvation: anterior animals. After the rapid decrease in CO_2 production during the first few days of starvation, there is a period of at least several weeks during which a slight further decrease occurs, but sooner or later an increase appears. This increase appears earlier at higher than at lower temperatures, earlier in anterior than in posterior animals and earlier in animals which have undergone a second fission and regulation than in those which have undergone fission and regulation only once. The data for anterior animals follow in table 3.

In table 3 lot I is in all cases the earlier, lot II, the later stage of starvation. The table gives the number of days since last feeding, the length of the worms at the time of pH determination, the number of worms in each lot, the total weight of each lot, the number of comparative deter-

TABLE 2

Early stages of starvation

SERIES	DAYS SINCE LAST FEEDING	HEADS	NUMBER OF WORMS	WEIGHT	TIMES OF RECORD IN HOURS AND pH										
					0	1½	2	2½	3	3½	4	5	5½	6	7½
				grams											
654A I	1	Absent	4	0.0237	8.0		7.8−			7.5+				7.4−	
654A II	8	Absent	5	0.0271	8.0		7.8			7.6+				7.4+	
655 I	2	Absent	4	0.0257	8.0		7.8						7.5−		
655 II	9	Absent	5	0.027	8.0		7.9						7.6−		
656 I	1	Absent	4	0.0277	8.0			7.7			7.5				7.3−
656 II	12	Absent	5½	0.0288	8.0			7.8−			7.6				7.4
643 I	½	Present	4	0.0283	8.0	7.7+			7.6−			7.4			
643 II	3	Present	4	0.0284	8.0	7.8−			7.6			7.4+			
643 III	7	Present	5	0.0293	8.0	7.8			7.6+			7.5			
642 I	1	Present	4	0.0267	8.0		7.8+			7.7		7.6−			7.4−
642 II	6	Present	5	0.0269	8.0		7.9			7.8		7.7			7.5

minations made in each series and the pH readings for several hours. The color comparisons were made with great care, the two lots of each series being compared both with each other and with the standard tubes of the colorimeter in uniform light. Where repeated compari-

TABLE 3

Anterior animals 12 to 13 mm. at beginning of starvation. Heads removed before weighing

SERIES	DAYS SINCE FED	LENGTH	NUMBER OF WORMS	WEIGHT	COMPARISONS WITH SAME LOTS	TIMES OF RECORD IN HOURS AND pH					
						0	1	2	3	4	5
		mm.		*grams*							
699 I	15	11–12	4	0.0232	First I	8.1		7.9	7.7+	7.5	
699 II	77	8	9	0.0221	First II	8.1		7.95	7.8+	7.7+	
					Second I	7.6	7.5−			7.3	
					Second II	7.6	7.5			7.4−	
699A I	38	9	4	0.0156		7.7	7.63		7.53	7.45	7.38
699A II	100	6	9	0.0163		7.7	7.6		7.5	7.4	7.33
					First I	7.8	7.7+	7.62			
					First II	7.8	7.7	7.58			
705 I	36	8–9	4	0.0186	Second I	7.7	7.65	7.55		7.38	7.33
705 II	98	6	9	0.0187	Second II	7.7	7.65−	7.53		7.36	7.3
					Third I	7.7	7.62	7.55	7.48		
					Third II	7.7	7.6	7.53	7.45		
					First I	7.7	7.65	7.58			
					First II	7.7	7.62	7.55			
713 I	57	7–8	3	0.0119	Second I	7.7		7.6		7.5	7.45
713 II	119	5	7	0.0122	Second II	7.7		7.6		7.48	7.43
					Third I	7.6	7.5	7.44	7.4	7.36	
					Third II	7.6	7.5	7.42	7.38	7.34	

sons were made with the same lots, clean tubes and fresh indicator solution were of course used, and a different pH was used as starting point in at least one of the repetitions in each series, as the table shows (see pH under 0 hours). The second decimal place in the pH readings is estimated, not exact, and serves chiefly to indicate whether the difference

between the two lots of a series is great or small, but in no case was a difference recorded unless it was perfectly distinct and observed in all of several comparisons made within two or three minutes with the tubes in different relative positions. Many of the records were confirmed independently by other persons. The transfer of the worms individually in weighing makes the use of small numbers desirable in order to avoid long exposure of some animals to the air, and this of course makes the differences in pH smaller than they would be if large numbers were used.

In series 699 of the table, lot I with fifteen days starvation produced CO_2 more rapidly than lot II with seventy-seven days of starvation in both of the two tests made. After these tests the worms of these two lots were returned to water and allowed to starve for twenty-three days more, during which time they regenerated heads in place of those cut off. At the end of this time the heads were removed again, the lots weighed and another pH determination made, the data for which are given in series 699A. At this time the later stage of starvation (lot II) produces CO_2 more rapidly than the earlier (lot I), i.e., the relative rates are the reverse of those in series 699. Moreover, taking into account the loss of nearly one-third in weight between the time of series 699 and 699A, the change in pH, though not directly comparable in the two series because of different starting points, indicates that the rate of CO_2 production of lot II is higher in 699A than in 699. These two series, which show earlier and later conditions in the same two lots, afford evidence in two ways that the rate of CO_2 production increases during the later stages of starvation. The great loss of weight during the twenty-three days between the two series is of course due not merely to starvation, but also to the fact that during this period the animals regenerated new heads, which were removed before the second weighing.

In series 705, lot I, starved thirty-six days, shows less rapid change in pH than lot II, starved ninety-eight days, all three tests giving the same comparative results. In series 713 still more advanced stages of starvation are used, lot I, fifty-seven days, lot II, one hundred and nineteen days, and again the more advanced stage shows the more rapid change in pH in all three tests.

From other starvation stocks started at other times and from other general stocks of worms three series of comparative pH determinations like those of table 3 were made, the starvation periods being thirty-nine and seventy-eight, forty and seventy-nine, forty-three and eighty-two

days. In two of these series the more advanced stage showed the more rapid change in pH, in one a slightly less rapid change than the less advanced stage. In these series the heads were not removed and it is possible that differences in motor activity may have been responsible for the exceptional result in the one series. Unfortunately it was impossible to carry on to later stages the stock from which these series were taken.

The more important data on anterior animals are briefly summarized as follows: A total of thirty-one animals, starved thirty-eight to fifty-seven days, was compared in lots of equal or nearly equal weight with a total of seventy animals, originally the same size, but starved seventy-seven to one hundred and nineteen days. Ten comparative pH determinations were made with six pairs of lots, each determination comprising two or more pH records at intervals of one to three hours. In nine of these determinations (25 and 55 animals) the more advanced stage of starvation showed a distinctly more rapid change in pH. In one determination (6 and 15 animals) the more advanced stage showed a slightly less rapid change in pH, but in this series the starvation periods were such that no very great differences were to be expected and since the heads were present differences in motor activity may have affected the result. No other exceptional cases have been observed with starvation periods of forty and eighty days or more in the two lots.

The later stages of starvation: posterior animals and products of second fission. Most of the posterior animals resulting from the first fission or section (p. 239) at the beginning of starvation undergo a second fission and regulation during the first few weeks of starvation, so that in the later stages of a starvation stock, instead of posterior animals, one finds mostly anterior-of-posterior and posterior-of-posterior animals (p. 238). Sufficient material has, however, been found in my stocks for four comparative series of earlier and later stages with posterior animals, though none represent very late stages of starvation. In these series lot I, six worms in each series, a total of twenty-four worms, had been starved thirty-nine to forty-three days and lot II, eleven, eleven, nine and ten worms, a total of forty-one worms, had been starved seventy-eight to eighty-two days, i.e., about twice as long as lot I. In each series the earlier stage, lot I, showed a more rapid change in pH than the later stage, though in one series the difference was very slight. In these posterior animals then the rate of CO_2 production is still higher in the earlier than in the later stage after these starvation periods, while in anterior animals (see preceding section) with corresponding starvation

periods, the rate of CO_2 production is almost always higher in the later than in the earlier stage.

For the still more advanced stages of starvation it was necessary to use the products of the second fission of posterior animals. These of course have used a part of their body substance in a second regulation as well as in starvation, consequently at any given time the products of the second fission of posterior animals represent a more advanced stage of starvation than animals which have starved for the same length of time, but have not undergone a second fission and regulation. The chief data obtained with these groups of animals are given in table 4.

Table 4 is similar in plan to table 3, except as regards the second column, in which the origin of the animals of each lot as regards preceding fissions is given. In series 714 and 700 posterior animals (I) are compared with posterior products of a second fission (II, posterior-of-posterior), the period of starvation of both lots being in series 714, fifty-nine days, in series 700, seventy-eight days. In both series, lot II, the posterior-of-posterior animals, shows the more rapid change in pH. In series 714 four comparisons were made, but since the result was the same in all only two are given in the table. In series 700 only two comparisons were made. Series 697 gives data for posterior (I) and posterior-of-posterior animals (II), starved seventy-seven days, the latter showing the more rapid change in pH.

Series 706 and 712 are both comparisons of posterior-of-posterior animals in earlier (I) and later stages (II) of starvation. In series 703, where the two lots have been starved respectively thirty-seven and ninety-nine days there is no difference in the first comparison and a very slight difference in the second. In series 712 with fifty-seven and one hundred and nineteen days of starvation the later stage shows the higher rate of change in pH in all three comparisons.

This table shows then, first, that after two months or more of starvation, products of a second fission of posterior animals show a higher rate of CO_2 production than the posterior animals themselves; and second, that posterior products of second fission (posterior-of-posterior) in sufficiently advanced stages of starvation possess a higher rate of CO_2 production than in earlier stages.

In another series not tabulated anterior-of-posterior animals were compared at twenty-three and eighty-five days of starvation, the earlier stage showing a very slightly more rapid change in pH than the later. Heads were not removed in this case and it was recorded at the time of experiment that the earlier stage showed distinctly more motor activity

TABLE 4

Posterior animals and products of fission of posterior animals: length at beginning of starvation 12 to 13 mm.; heads removed before weighing except in series 700

SERIES	ORIGIN	DAYS SINCE FED	LENGTH	NUMBER OF WORMS	WEIGHT	COMPARISONS WITH SAME LOTS	TIMES OF RECORD IN HOURS AND pH							
							0	1	2	3	4	5	6	7
			mm.		*grams*									
714 I	Post.	59	9–10	3	0.0123	First I	7.6	7.55			7.45			7.38
714 II	Ant.-of-post.	59	5–6	13	0.0124	First II	7.6	7.55			7.43			7.35
						Second I	7.6				7.44	7.4		
						Second II	7.6				7.42	7.38		
700 I	Post.	78	8–9	4	0.0136	First I	7.6	7.5		7.42		7.35		
700 II	Ant.-of-post.	78	5–6	11	0.0138	First II	7.6	7.5−		7.37		7.3		
						Second I	7.8	7.75				7.6		
						Second II	7.8	7.73				7.52		
697 I	Post.	77	8–9	4	0.0134	First I	8.1	8.1−			7.95		7.9	
697 II	Post.-of-post.	77	6–7	10	0.0131	First II	8.1	8.0+			7.9		7.8	
						Second I	7.6	7.58				7.4	7.37	
						Second II	7.6	7.55				7.37	7.33	
706 I	Post.-of-post.	37	8	4	0.0136	First I	7.7	7.6+			7.38	7.32		
706 II	Post.-of-post.	99	6	10	0.0130	First II	7.7	7.6+			7.38	7.32		
						Second I	7.7	7.65			7.49			
						Second II	7.7	7.65+			7.5			
						First I	7.7	7.6	7.55					
						First II	7.7	7.57	7.5					
712 I	Post.-of-post.	57	7–8	4	0.0127	Second I	7.7		7.6			7.48		
712 II	Post.-of-post.	119	5	10	0.0124	Second II	7.7		7.6			7.45		
						Third I	7.6	7.52		7.43	7.38			
						Third II	7.6	7.52		7.42	7.36			

than the later, consequently the result is of doubtful value. No other exceptions have been found.

Miscellaneous data. From other starvation stocks starved at a higher temperature and therefore more rapidly than those used in the preceding series, anterior animals, 10 to 11 mm. in length after twelve days of starvation showed a markedly lower rate of CO_2 production than anterior animals (or perhaps products of second fission of anterior animals) 5 to 7 mm., after forty-seven days of starvation. In another series from a stock starved forty-two days, the largest anterior animals (8 mm.) showed a lower rate of CO_2 production than the smallest anterior animals (5 to 6 mm., perhaps products of second fission). Here the smaller animals, either because of a second fission and regulation or because of accidental conditions (p. 238) represented a more advanced stage of starvation showed a higher rate of CO_2 production than the larger.

After eighty days of starvation anterior animals show a distinctly higher rate of CO_2 production than posterior animals with the same starvation period. In view of the data already presented this difference is to be expected from the fact that anterior animals undergo more rapid reduction than posterior animals.

In still another experiment anterior and posterior animals from a stock starved forty-three days were cut in two at the mouth and equal weights of the posterior pieces of each were compared. The posterior region of the anterior animals has of course been concerned in the formation of a new posterior end since starvation began, while that of the posterior animal has not. It was also noted above (p. 237) that the anterior animal undergoes reduction more rapidly than the posterior in starvation, and figures 4 (anterior) and 5 (posterior) indicate the difference in intestinal volume of the posterior region of the two animals. The posterior pieces of the anterior animals showed a distinctly more rapid change in pH than the posterior pieces of the posterior animals. In other words, the pieces in which the alimentary tract was most reduced showed the higher rate of CO_2 production, although starved for the same number of days as the other pieces.

In one series anterior animals starved one hundred and thirty-four days, without fission during starvation, showed a less rapid change in pH than posterior-of-posterior animals starved seventy-two days, both lots being about 6 mm. in length. In this case the anterior animals had been in the biometer (see p. 250) preceding the colorimetric test and may have been injured by exposure to the air, or possibly by mer-

cury vapor, such injury having been observed in other cases in the biometer. This is the only exceptional case in this group of data, and it is at least of doubtful value.

Starved and fed animals. The chief purpose of the present paper is to present the data concerning the conditions during starvation. Some comparative work has been done on CO_2 production in starved and fed animals and on the effect of feeding after periods of starvation, but the data are not yet as complete as is desired, and their presentation in full is postponed to another time. A few characteristic results among those already obtained along this line are briefly stated to show what has already been determined along this line, and as a basis for discussion of certain questions.

Direct comparison of pH change in the advanced stages of starvation with that of large, well-fed animals by the method described above presents difficulties because the large number of starved animals necessary to equal in weight even one or two of the large ones cannot be handled rapidly enough in weighing to avoid danger from drying. Other methods of procedure are more satisfactory. A few direct comparative experiments have, however, been made in which pieces of the large animals including the region between head and mouth, the head being removed, have been used instead of the whole animals. Animals starved one hundred and twenty-three days and reduced by starvation and fission from 25 mm. to 4 or 5 mm. produce CO_2 more rapidly than pieces of well-fed animals of the original length, i.e., 25 mm. and at about the same rate as pieces of well-fed animals 15 to 16 mm. which show a somewhat higher rate than the pieces of 25 mm. animals. Anterior animals from another stock, starved one hundred and thirty-seven days without fission during starvation, and reduced from 15 mm. to 4 or 5 mm., showed in one series a distinctly higher rate than pieces from 25 mm. well-fed animals, while in another series where only a single piece of an old animal and three starved worms were used, the rate was somewhat lower in the starved animals than in the fed piece. In general the data indicate that after four months or more of starvation, the rate of CO_2 production is actually higher than, or at least about equal to the rate at the beginning of starvation, i.e., the decrease in rate during the early stages of starvation has been balanced or more than balanced by an increase in rate during the later stages. This result agrees with and confirms the data of preceding sections, which show an increase in rate in the later stages of starvation.

It is a familiar fact that planarians which have been reduced by starvation to a small fraction of their original size, will begin to grow again when fed and may again attain the original size and again be reduced by starvation. So far as our knowledge goes, this alternation of growth and reduction may be repeated indefinitely with proper food conditions. According to criteria thus far applied, animals which are fed after a period of starvation and reduction are physiologically younger than they were at the beginning of starvation and are comparable to well-fed animals of the same size as themselves, as regards bodily proportions, rate of growth, susceptibility to various agents, capacity for acclimation (4), (6, chap. vii). As feeding is continued and increase in size again occurs, rate of growth, susceptibility and capacity for acclimation undergo gradual decrease, as in other animals undergoing growth and physiological senescence, so that when the animals again attain the original length, e.g., 25 mm., their condition in these respects is not appreciably different from the condition before starvation. The data at hand concerning the effect on CO_2 production of feeding after starvation confirm the earlier conclusions.

It was shown in table 2 that even a few days of starvation brings about a marked decrease in CO_2 production. It has been found that a single feeding after such a short period of starvation brings the animal back almost to its original condition, and that after two or three feedings at intervals of a day or two its rate of CO_2 production is the same as or slightly higher than that of fed animals of the same size. For example, animals starved eleven days and then fed once with liver twenty-four hours before comparison, show a rate almost as high as that of animals of the same size fed three times a week, and very much higher than that of animals starved twelve days. A second feeding twenty-four to forty-eight hours after the first is usually sufficient to establish the original rate.

After longer periods of starvation more food is required to raise the rate of CO_2 production to the level of that of well-fed animals of the same size. For example, animals starved eighty-seven days and reduced by starvation, fission and regulation from 25 mm. to 4 or 6 mm., after feeding three times on three successive days, show a rate of change in pH almost midway between that of young well-fed animals 5 to 6 mm. and animals 4 to 6 mm. starved ninety days without feeding. Animals starved eighty-seven days and reduced from 25 mm. to 4 or 6 mm., after feeding seven times in eight days show a rate of CO_2 production as high as, or slightly higher than that of young, well-fed animals of the

same size. Three feedings are not sufficient to bring these animals up to the level of young, well-fed animals of the same size, but seven feedings accomplish the result and if feeding is continued, the animals from this time on are indistinguishable in any way, so far as we know at present, from the young, growing animals, which have not been starved. As regards rate of CO_2 production as well as other physiological characteristics, the animals which have been starved and then fed have undergone a physiological rejuvenescence, the degree of rejuvenescence depending on and varying directly with the degree of starvation and reduction.

Comparative CO_2 estimations with the Tashiro biometer. In the earlier work on starvation in Planaria a few data obtained with the Tashiro biometer (10, Appendix) were presented (4, p. 434). According to these data, animals starved ninety-four days showed a rate of CO_2 production about equal to that of young fed animals of the same size.

These earlier data have now been supplemented by some further work with the same apparatus. In these later estimations single headless pieces from the anterior body region of well-fed worms 25 mm. in length were compared with an approximately equal weight of headless animals reduced by starvation, fission and regulation from 25 to 6 or 7 mm. In ten estimations with differences in weight between fed and starved not exceeding 14 per cent with the excess in different sides in different cases, the starved animals showed in every case a distinctly higher rate of CO_2 production than the old fed pieces.

Several comparisons between animals starved one hundred and twenty to one hundred and thirty days and young fed animals of the same size gave a higher rate of CO_2 production for the starved than for the fed animals. These results do not agree with those of the colorimetric determinations.

Work with the biometer was discontinued because I came to believe that the method was not suitable for this material. The animals are more or less stimulated by handling and the starved animals are usually distinctly more susceptible than the fed and react more strongly to such stimulation. With the biometer it is necessary to make the estimations as soon as possible, i.e., within five or ten minutes after the animals are placed in the chambers, because even the headless animals usually become restless after a time on account of exposure to the air, although the atmosphere of the chambers is saturated with water vapor, or for some other unascertained reason. Consequently the carbon dioxide estimation is made before the animals have recovered from the stimulation due to the transfer and since there is every indication that

the starved animals are more stimulated than the fed the biometer is likely to show too high a rate of CO_2 production for the starved animals. That this is the case was proved for some series by making a colorimetric comparison with the animals used in the biometer. Starved animals which showed in the biometer a higher rate than the fed showed a rate equal to or lower than that of the fed animals by the colorimetric method. With the latter method the determination extends over several hours and the headless animals usually do not move at all after the first few moments, if the tubes are properly handled. There is good reason to believe, therefore, that the colorimetric data are more nearly correct than those obtained with the biometer, but if the rates as shown in the biometer result from a greater degree of stimulation of the starved animals the reason for their greater sensitiveness or more intense reaction remains to be explained. The following discussion will throw some light on this point.

DISCUSSION

The colorimetric determinations indicate that the rate of CO_2 production falls rapidly and to a marked degree during the first few days of starvation, then more slowly during several weeks, and that this decrease is followed sooner or later by an increase in rate which may balance or even overbalance the earlier decrease, so that in advanced stages of starvation the rate of CO_2 production may be as high as, or higher than that at the beginning of starvation. That the condition of the alimentary tract is an important factor in these changes is indicated by several facts: first, the rapid decrease during the first few days of starvation can be compensated and the animal brought back to the original level by a single feeding; second, the decrease in rate apparently continues as long as any appreciable amount of nutritive reserve is present in the cells of the alimentary tract, while in the period of increase in rate the animal is apparently subsisting entirely upon its own protoplasm and the alimentary tract is reduced more rapidly than the body as a whole; third, in animals starved for the same length of time, those in which the alimentary tract is most reduced show in general the higher rate of CO_2 production; fourth, even after several months of starvation, a few feedings bring the rate of CO_2 production up to that of young fed animals of the same size as the starved animals, i.e., to a level far above that at the beginning of starvation. The metabolism of the alimentary tract evidently plays a large rôle in the total CO_2 production of well-fed animals, but it is also evident that this metabolism decreases rapidly

in the absence of food and increases again when food is given. This is to be expected since the alimentary tract is functionally concerned with food, and in the absence of food its functional activity must decrease. Undoubtedly some functional activity is concerned in the transformation and removal of the nutritive reserves from the cells of the alimentary tract, but as starvation progresses and the reserves gradually disappear, even this decreases and sooner or later the alimentary tract becomes a practically functionless, quiescent organ and under these conditions autolysis in its cells probably increases and it becomes itself a source of nutrition for other parts.

The other essential organs of the body, ectoderm, muscles, nervous system, etc., do not undergo any such loss of function in starvation as does the alimentary tract. Their functional activity is maintained or even increased, since the starved animal becomes more active. All these facts indicate that the decrease in CO_2 production during starvation in Planaria is at least in large measure the result of a decrease in the metabolic activity of the alimentary tract. Probably the period of most rapid decrease at the beginning of starvation coincides with the period of disappearance of food from the tract, and the later period of slow decrease with the period of mobilization of reserves.

The increase in rate of CO_2 production during the later stages of starvation and the fact that starvation followed by feeding accomplishes a rejuvenescence as regards rate of CO_2 production and various other characteristics, remain to be accounted for. It is in this connection that the significance of the earlier work on susceptibility in relation to starvation appears (4), (6, chap. vii). These earlier investigations showed that the susceptibility of Planaria to lethal concentrations of KNC and various other toxic agents increases from the beginning of starvation and that animals reduced by starvation show about the same or a somewhat higher susceptibility than fed animals of the same size. It was found, however, that the capacity for acclimation decreases during starvation but is increased above the original capacity by feeding after starvation. The conclusion drawn from these earlier experiments was that the rate of oxidation underwent an increase during starvation so that the animals became physiologically younger in this respect as starvation progressed, but since the capacity for acclimation decreases during starvation, while it is greater in young than in old animals, it was suggested that nutritive condition as well as rate of oxidation is a factor in acclimation, and that the process of rejuvenescence is not complete in all respects until after the starved animal has been fed.

Although the same general conclusion, viz., that starvation followed by feeding accomplishes a process of rejuvenescence is drawn from the data on susceptibility and those on CO_2 production, an apparent discrepancy exists in the data obtained by the two methods concerning what occurs during starvation. Susceptibility increases from the beginning of starvation, and at the end of a starvation period is at least equal to that of fed animals of the same size as the starved, and is not further increased by feeding, while CO_2 production decreases, at first rapidly, then more slowly and sooner or later begins to increase, but is increased still further by feeding after starvation.

This apparent discrepancy in the results of the two methods depends, at least very largely, upon the fact that in the earlier experiments susceptibility was always determined by survival time of the ectoderm and body wall, no account being taken of the condition of the alimentary tract, while the data on CO_2 production concern total CO_2 production, that of the alimentary tract as well as that of the ectoderm and body wall. In the earlier work on susceptibility it was observed that in lethal concentrations the alimentary tract of fed animals disintegrated almost as early as ectoderm and body wall, while in starved animals the alimentary tract might remain completely intact for some time—depending on the concentration—after ectoderm and body wall were completely disintegrated or cytolyzed. The significance of these facts was not recognized until the work on CO_2 production made it clear. Since then the experiments on susceptibility have been repeated many times, not only with KNC but with various other agents. This later work shows that the susceptibility of ectoderm and body wall increases from the beginning of starvation, while the susceptibility of the alimentary tract, not only as compared with that of the body wall, but as compared with that of the alimentary tract at the beginning of starvation decreases during the earlier period of starvation, but in the advanced stages increases, though it remains below that of the body wall until the animals are fed, when it soon attains the relation characteristic of fed animals. These experiments were made with intact animals, where the chemical agents must pass through the body wall to reach the alimentary tract, but they show the changes in relative susceptibility of tract and body wall and the differences in susceptibility of the tract at different times. Where directly exposed at cut surfaces, the functionally active alimentary tract of fed animals is fully as susceptible as the body wall, that of starved animals less susceptible than the body wall.

It is evident then that when alimentary tract as well as body wall is taken into account in determining susceptibility, the data on susceptibility and those on CO_2 production are not in conflict, but supplement and confirm each other. Taking into account all the facts at present available, the following interpretation suggests itself: the ectoderm and body wall, which maintain their functional activity and subsist in part upon their own substance from the beginning, show a gradual increase in rate of oxidation during starvation, while the rate of oxidation in the alimentary tract undergoes rapid and marked decrease in the absence of food and probably still further decrease more slowly, as the reserves are used. Alimentary tract oxidation in well-fed animals is so large a part of total oxidation that the decrease in activity of the alimentary tract in the absence of food brings about a decrease in total CO_2 production, in spite of the probable increase in CO_2 production in ectoderm and body wall. Gradually, however, the alimentary tract approaches a minimum of oxidative activity, and as its substance is used, its rate of CO_2 production also begins to rise. Somewhere in the course of these changes increase in total CO_2 production begins to appear. In advanced stages of starvation the ectoderm and body wall are functionally active, while the alimentary tract is quiescent, consequently the animal is younger as regards susceptibility of ectoderm and body wall than as regards total CO_2 production. Feeding after starvation alters the condition of ectoderm and body wall but little, except gradually, as growth occurs again, consequently susceptibility of these parts is but little affected: but feeding increases greatly the metabolic activity of the alimentary tract and so increases total CO_2 production to a marked degree.

This interpretation throws no further light upon the question of the increase in capacity for acclimation following feeding after starvation. It may be, as suggested earlier (4), that this capacity is associated in some way with nutritive condition, possibly in connection with the synthesis of protein molecules under the conditions to which acclimation occurs, so that capacity for acclimation does not increase until the animal is fed after starvation.

If this interpretation is correct, the earlier conclusion that starvation brings about a rejuvenescence as regards rate of oxidation holds good, but the process may be masked, at least as regards CO_2 production, during the earlier stages by the decrease in functional activity of the alimentary tract. In some way apparently the reentrance into metabolism of substances deposited in the cells during growth and differentia-

tion results in an acceleration of the fundamental reactions. We may interpret this change in terms of catalytic action and its products, of changes in molecular constitution, of aggregation of colloids or perhaps in other terms, but whatever these terms may be, it seems to be true that growth and differentiation are associated with a decrease, and reduction and dedifferentiation with an increase in rate of the fundamental reactions. As regards starvation, the process of rejuvenescence is not complete in all respects until the starved animal is again fed.

E. J. Lund (8) and B. L. Lund (9) have recently reported that during the starvation of Paramecium susceptibility to KNC increases while oxygen consumption decreases. On the basis of these observations they conclude that susceptibility to cyanides is simply a matter of permeability and independent of the rate of oxidation. As I have stated elsewhere (5) the susceptibility of Paramecium and various other infusoria to KNC is determined by ectoplasmic changes for it is impossible to determine with certainty when the entoplasm is affected or killed. Whether we take cessation of movement or disintegration of ectoplasm as the criterion, this is true, when the ectoplasm disintegrates the entoplasm flows out and when it dies cannot be determined with certainty. There is every reason to believe that in well-fed Paramecium, as well as in Planaria, ectoplasmic oxidation is only a small fraction of the total and that in the absence of food a marked decrease in oxidation occurs in the entoplasm. This decrease doubtless determines the decrease in total oxygen-consumption observed by Lund while conditions in the ectoplasm, which corresponds functionally to ectoderm and body wall of Planaria, bring about the increase in susceptibility. In fact the Lunds' data as far as they go are in complete agreement with my own investigations on Planaria, and as I have pointed out above, the facts indicate that the discrepancy between the data on susceptibility and those on metabolism is only apparent. Their conclusion that susceptibility to cyanide is independent of rate of oxidation is therefore not at present justified, since their own data are not complete as regards the difference in condition between ectoplasm and entoplasm, and in the light of all the facts, the opposite conclusion seems the only one possible. Lund presents no data on stages of starvation in Paramecium comparable to the late stages of starvation in Planaria, perhaps because Paramecium being a single cell, dies before such stages are attained. Nevertheless, the fact that his results on starvation obtained with a different organism and in part by a different method, agree as far as they go with

my own is significant.[1] Data on susceptibility to lack of oxygen and on oxygen consumption during starvation in Planaria will be presented in later papers.

SUMMARY

1. CO_2 production decreases rapidly during the first few days of starvation in Planaria and continues to decrease more slowly during several weeks, but in advanced stages of starvation it increases.

2. The more rapidly starvation, and particularly the reduction of the alimentary tract occurs, the more rapidly these changes in CO_2 production take place. Fission and regulation accelerate the progress of starvation.

3. After three or four months of starvation CO_2 production is at least equal to or greater than that at the beginning of starvation.

4. Though the work is not yet completed, the data already obtained upon the effect of feeding after periods of starvation show that feeding after a few days of starvation raises the CO_2 production to the original level, while feeding after long periods of starvation and reduction raises the CO_2 production approximately to the level of that in young fed animals of the same size as the starved, reduced animals. In other words, the animals reduced by starvation and then fed are physiologically younger than at the beginning of starvation, and if feeding is continued are capable of undergoing growth and physiological senescence again from the stage represented at the end of the starvation period.

5. Susceptibility of the ectoderm and body wall increases from the beginning of starvation while susceptibility of the alimentary tract at first decreases very markedly and later increases. The apparent disagreement between the results of the earlier susceptibility data and the data on CO_2 production is accounted for by the great decrease in functional metabolism in the alimentary tract in the absence of food, while no such decrease occurs in body wall and ectoderm. In the earlier work susceptibility was measured in terms of ectoderm and body wall alone, but when the susceptibility of the alimentary tract is taken into account, as

[1] Several months before the publication of his paper Lund was informed in personal correspondence of the results of my work on starvation in Planaria and the interpretation suggested by these. In this correspondence it was pointed out that the apparent discrepancy between the earlier results of the work on susceptibility and that on CO_2 production was accounted for by the fact that susceptibility was measured in terms of ectoderm and body wall, while the data on CO_2 production concerned total CO_2 production.

in later work, the results of the susceptibility method supplement and confirm the data on CO_2 production. All the facts at present known indicate that, except for the decrease in functional metabolism in the alimentary tract, the rate of oxidation increases during starvation in Planaria.

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THE FUNCTION OF THE NUCLEUS OF THE LIVING CELL

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METHOD

Of the different methods which have been employed to solve the problem of the function of the cell nucleus, the one which has given the most illuminating results is the method of extirpation: removing the nucleus and comparing the enucleated cell with a normal nucleated cell. In order that the nucleus may be removed from a cell or that a cell may be cut in half, it is necessary or at least expedient that a cell of considerable size be selected; but at the same time it must be a cell which can be kept alive or cultivated for several generations, if necessary. The cells which meet these requirements most exactly are certain unicellular organisms, certain protozoa. Among such a large and diverse group of organisms it is not difficult to find one which is peculiarly suited for the problem at hand. For the experiments which follow, Ameba proteus was selected. Owing to the slow movements and absence of shell in this organism, it can readily be cut in half; or if desired the nucleus may, after some practice, be removed with as little as one-tenth or less of the cytoplasm. Moreover, the lack of differentiation in Ameba is a decided advantage for we are certain that in removing part of the cytoplasm with the nucleus we are removing no important organ of the cell. The one disadvantage in the use of this organism is the difficulty of cultivating it. Like all rhizopods, amebas are difficult to raise; but many investigators have succeeded in keeping healthy cultures in the laboratory for years.

In view of the difficulties which are so often met in cultivating amebas, it may not be out of place to add one more method to those which have been described for rearing these valuable experimental animals. It is almost literally true that each investigator has a method of raising amebas which no one can use except himself. This is not surprising when it is seen that "water" is employed in making the cultures, with

little regard for its precise character. While amebas may flourish in the water of one locality, they may quickly die in the water of another locality. This difficulty was avoided by the use of distilled water.[1] The method which follows is based upon one which has been employed by Miss Hyman of the University of Chicago.

A small amount of hay, including both stalks and leaves, is cut into pieces about three inches long; 4 gm. of this material are then placed in a large beaker. To this is added 0.2 gram of dry bread crumbs and 500 cc. of distilled water. The material is boiled for a few minutes and then poured into flat dishes to a depth of 1 to 2 cm. The dishes are covered to keep out dust, and if water evaporates from them it can be replaced by distilled water. After the infusion has cooled, several pipettes full of fluid are added from a culture which contains healthy amebas or from a pond or stream where amebas are found. In doing this care is taken to avoid taking up any of the sediment, for it is the sediment rather than the supernatant fluid which is apt to contain enemies to Ameba. This inoculation may be repeated. In the course of two weeks the culture becomes cloudy with bacteria, zoöglea and small ciliates and flagellates, the food of Ameba. A small dish of the culture fluid is now seeded with amebas, care being taken to avoid large organisms such as crustacea and worms. If living amebas are not present a week later, more of the fluid containing amebas should be added. This culture is used to inoculate the rest of the infusion. The addition of a little hay occasionally will keep the amebas present in great numbers. The room should not be allowed to become very hot or very cold.

The amebas were transferred to different media by means of a fine glass capillary pipette, the sharp end of which had been made smooth by passing it through a flame. In most of the experiments the animals were kept in drops of fluid upon slides with a depression at each end. The slides were kept in a moist chamber in a room whose temperature did not vary greatly from 20°C. For cutting the amebas in half or cutting the nucleus away from the rest of the cell, a fine glass needle was drawn off at a slight angle from the end of a glass rod. The cells were divided under a binocular microscope.

Since the animals would not live long in the tap water of this city, a search was made at the outset for a solution in which they could be kept in healthy, active condition. Ten amebas were removed from a cul-

[1] The prejudice of many biologists against the use of distilled water and the prevalent notion that it is toxic, are not supported by experiments upon the toxicity of distilled water (Daniel, '08).

ture, freed from food material and debris and transferred to the solution to be tested. Each day they were examined and transferred to a fresh solution, the experiment being continued until all of the animals were dead. It appeared at first that distilled water was the most satisfactory medium that could be obtained. Thus ten amebas lived (without food) for an average of 13.2 days in distilled water, whereas animals kept in tap water, for example, soon assumed an abnormal appearance and could seldom be kept alive longer than one week. But it was later discovered that the distilled water was greatly improved by the addition of one-tenth the volume of spring water. Not only was the average life (14½ days) somewhat longer, but the condition of the animals during this time was greatly improved. In the distilled water the animals soon cease to be attached to the bottom and are hence unable to move from place to place in the normal manner; but in the "10 per cent spring water," after it became saturated with air, they remained attached up to a few days before death. The spring water used contained a trace of calcium, but not so much as was contained in the tap water. In the following experiments the amebas, unless otherwise noted, were kept in the 10 per cent spring water.

RELATION OF THE NUCLEUS TO MOVEMENT

The movement of normal amebas in a suitable medium, such as the dilute spring water, when placed upon a glass slide, was found to be of the limax type. The ameba is attached to the slide by means of some sticky secretion and flows steadily forward, sending out pseudopods at the anterior end, often from alternate sides. Frequently amebas are found which are not attached to the slide and such amebas may project pseudopods in any direction, but are unable to move from place to place. These animals will in time, however, adhere to the slide, unless they are abnormal or the medium is unsuitable. When these active amebas are stimulated roughly, as by pressure with a glass needle, they retract into a more or less spherical shape and small droplets of protoplasm may be seen projecting from the surface.

The effect of amputation of the nucleus upon the movement of the cell may be seen from the following typical experiment:

3.21 p.m. An active ameba which was attached to the slide was divided into two approximately equal parts, the nucleus remaining in the posterior half. Both parts went into the typical condition of stimulation.

3.23 p.m. Both pieces have put out pseudopods and are active, but the non-nucleated part, which was cut from the anterior end, is much more active than the nucleated and posterior part.

3.27 p.m. Both pieces are moving in the typical limax fashion, but the movement of the non-nucleated piece has become perceptibly slower.

3.28 p.m. The non-nucleated piece ceases its progressive movements and slowly retracts into a somewhat corrugated sphere. Only an occasional, very blunt pseudopod is now produced, and slight agitation shows that the fragment is no longer attached to the slide. The nucleated piece differs from a normal ameba only in size.

The retraction into the spherical shape is an invariable phenomenon and is of such a definite character that the time of its occurrence may be accurately determined. Thus in fifteen amebas which were so divided that the nucleus remained in the posterior half, the retraction occurred at an average of nine minutes after the operation. To the observer it resembles strongly the response to stimulation, except for the absence of the protoplasmic droplets mentioned above. The protoplasm is capable of movement, as is shown by transferring it to another medium such as distilled water, to which it responds by a change of shape.

This effect of amputation of the nucleus upon movement is not always permanent. If a number of amebas are cut in half and the nucleated and non-nucleated pieces observed each day, it will be noticed on the first or second day after the operation that many of the non-nucleated fragments are moving in the typical limax fashion, and in fact there is sometimes no great difference between the movements of the nucleated and the non-nucleated pieces. Slight agitation, however, shows that the non-nuclear amebas are scarcely attached to the slide; the slightest disturbance serves to dislodge them. This fact, which must be due to the failure to produce the sticky secretion, is probably partly responsible for the difference in movement between the nuclear and the non-nuclear parts.

The fact that a non-nucleated ameba may, under any conditions, exhibit normal movements, justifies us in concluding that the nucleus is not necessary for movement. In Ameba, however, movement is affected in some indirect way by removal of the nucleus.

THE SURVIVAL OF THE NON-NUCLEATED CELL

All observations made upon cells which have been deprived of their nuclei prove conclusively that non-nucleated protoplasm is destined to die without growing or dividing. But in most cases, particularly in

Ameba, it has been impossible to get this protoplasm to take in food, and even that food which is already included in the protoplasm is but imperfectly digested. Consequently it is fairer to compare the survival of an enucleated cell with one which has been isolated without food rather than with an actively feeding cell. When this is done it is found that the non-nucleated cell lives almost as long as the nucleated one, *provided there is no difference in size.* If an ameba is cut into unequal parts, the nucleus, owing to its central position, will usually be found in the larger part. Since large fragments live longer than small ones, special care must be taken to avoid a difference in size.

MEDIUM	SURVIVAL OF NUCLEATED PART	SURVIVAL OF NON-NUCLEATED PART
	days	*days*
Distilled water	7	6
Distilled water	7	7
Distilled water	10	6
Distilled water	9	4
Distilled water	7	4
Distilled water	5	3
Distilled water	9	6
Culture water	7	5
Culture water	8	6
Culture water	6	6
Culture water	9	7
Culture water	11	8
Culture water	7	8
Culture water	7	7
Culture water	7	7
Average	7.7	6

Fifteen amebas were cut in half and both halves were kept without food in either distilled water or in water taken from the culture. The number of days which each piece lived after the operation is given in the above table.

It will be noted that in some cases the non-nucleated fragments lived as long as those which contained nuclei. In fact by cutting the ameba in such a way that the piece containing the nucleus was smaller than the piece which lacked it, the non-nucleated piece could be made to outlive the nucleated one. Thus ten amebas were divided in such a way that the nucleated piece was from one-half to one-fourth the size of the other piece. The results are tabulated below:

	SURVIVAL OF NUCLEATED PART	SURVIVAL OF NON-NUCLEATED PART
	days	*days*
	6	10
	7	7
	3	13
	6	5
	3	6
	6	7
	7	7
	6	8
	7	7
	6	10
Average	5.7	8

We may conclude that a cell deprived of its nucleus may survive as long as a cell deprived of food.

THE NUTRITION OF AMEBA; THE USE OF SUBSTANCES IN SOLUTION

The apparent resemblance of enucleated amebas to amebas deprived of food suggested that some of the phenomena exhibited by the non-nucleated cell might be the result of starvation and have no direct connection with the absence of a nucleus. Accordingly, an attempt was made to supply the amebas with adequate food substances. Since it was not possible to get the enucleated organisms to take in food particles, an effort was made to provide a medium in which amebas could be nourished by the absorption of substances in solution.

It has been pointed out (Burrows and Neymann, '17) that no artificial synthetic medium has ever been provided which was adequate to nourish an animal cell although the blood and lymph apparently constitute a natural synthetic medium for the cells of higher organisms. Even cells cultivated in vitro depend upon the autolysis of neighboring cells for their nourishment, and the attempt to cultivate protozoa in nutritive organic media has met with complete failure (Biedermann, '16; Doflein, '11). In the light of recent experiments in nutrition, however, it seems possible that such negative results may be due to the failure to supply certain substances which are necessary for proper nutrition. An attempt was therefore made to provide an adequate medium for the primitive animal cell, Ameba proteus.

Since amebas were found to move more normally and to live longer in

distilled water to which had been added one-tenth the volume of spring water, this "10 per cent spring water" was used in making all solutions. Various carbon compounds, including several sugars, were now tested with the hope of finding a source of energy.

DATE	NUMBER OF AMEBAS LIVING		
	Water	1 per cent glucose	1 per cent levulose
February 7	20	20	20
February 8	20	20	20
February 9	18	20	20
February 10	15	20	20
February 11	15	20	20
February 12	15	20	20
February 13	13	20	20
February 14	13	20	20
February 15	13	20	20
February 16	13	20	19
February 17	13	20	19
February 18	13	20	18
February 19	13	20	18
February 20	11	18	17
February 21	11	17	17
February 22	11	17	15
February 23	9	17	15
February 24	7	17	15
February 25	6	16	14
February 26	6	16	12
February 27	4	12	11
February 28	3	12	8
March 1	2	11	8
March 2	2	10	2
March 3	2	6	0
March 4	1	4	
March 5	1	3	
March 6	1	0	
March 7	1		
March 8	0		
Average life	13.6 days	21.8 days	19.4 days

The method used was as follows: A number of amebas were selected from the same culture and freed from food and bacteria by transferring repeatedly to fresh solutions of 10 per cent spring water. They were then transferred to slides, placing ten in 10 per cent spring water

as a control, and ten in each of the solutions to be tested. The animals were examined and counted each day, washed in water and transferred to fresh solutions to prevent the development of bacteria. The most promising substances found were the hexoses, glucose and levulose, the effect of which may be seen in the preceding experiment.

DATE	NUMBER OF AMEBAS LIVING		
	1 per cent glucose	1 per cent glucose + 0.1 per cent urea	1 per cent glucose + 0.01 per cent NH_4NO_3
February 24	10	10	10
February 25	9	10	8
February 26	9	11*	8
February 27	9	12	8
February 28	6	12	8
March 1	5	12	8
March 2	5	13	8
March 3	4	12	8
March 4	4	12	8
March 5	4	12	8
March 6	4	10	8
March 7	4	10	8
March 8	4	10	8
March 9	4	8	7
March 10	4	6	7
March 11	4	6	7
March 12	4	6	4
March 13	3	5	3
March 14	2	4	2
March 15	1	3	2
March 16	1	3	1
March 17	1	3	0
March 21	0	1	
March 22		1	
March 27		0	
Average life	10.4 days	18.9 days	13.9 days

*The increase in number shown in this and subsequent tables is the result of the division of certain of the amebas.

Not only did the amebas live longer in the sugar solutions but during the early part of the experiment they were much more active. At the end of a week, however, many of the amebas began to take on an opaque appearance and ceased normal movements. The substance which was

formerly a food now acted as a poison. Child ('15) has shown that susceptibility to poisons increases during starvation.

Although these cultures were not sterile, it was possible to keep the bacteria from becoming numerous and thus prevent their becoming an appreciable source of food for the amebas.

Having found a carbohydrate food for Ameba, a search was now made for a source of nitrogen. In such a primitive cell the possibility that simple compounds of nitrogen may be used at once suggests itself. Of various compounds of nitrogen which were tried, the best results were obtained with ammonium nitrate and urea; and of these two, urea was much the better owing to its comparatively low toxicity. The effect of these substances is seen in the preceding experiment. (The comparatively short life of the animals is to be attributed to the use of an inferior culture.)

That these compounds are of some use to the amebas is indicated by the increased length of life when they are added to the solution; but that they are not an adequate source of nitrogen is indicated by the fact that the animals eventually die and that no growth can be detected. The shrinkage caused by the glucose would render the detection of growth difficult even if present.

Of special interest, however, are the three cases of cell division in the urea solution. Although hundreds of amebas have been kept under daily observation until they were completely disintegrated, no single case of cell division was ever observed unless food substances were supplied; and with two possible exceptions, no division was observed unless a source of nitrogen was added. On the other hand, divisions have been repeatedly observed in solutions containing urea or certain amino acids. That the division is not simply the result of the stimulating action of the urea upon the protoplasm is shown by the effect of urea solutions to which no glucose is added. In such solutions the amebas not only do not divide, but they die more quickly than in water alone. Urea in the absence of glucose is simply a mild poison but when urea and glucose are used together, the animals live longer, have a more normal appearance and may even reproduce. The necessity of using the two substances together indicates that they are built up or combined to form some more complex substance which is of use to the organism.

If this is a true case of organic synthesis, it would be interesting to see whether it occurs in the cell which has been deprived of its nucleus, in order to throw light upon the supposed synthetic function of the nucleus. This point is taken up later.

Having failed to obtain growth with simple compounds of nitrogen, the organisms were now supplied with amino acids. The use of simple amino acids was not promising. Some reproduction was obtained with a saturated solution of tyrosin, but the animals soon died. A mixture of amino acids was then prepared in the following way:

Five grams of Hammarsten's casein was heated for twenty hours with 100 cc. of a molecular solution of sulphuric acid. The hydrolysis was performed on a water bath in a flask fitted with a reflux condenser. At the end of the hydrolysis, when the solution no longer gave the biuret reaction, a saturated solution of barium hydroxide was added until the reaction was slightly alkaline, and the solution boiled to remove ammonia. Dilute sulphuric acid was added until the reaction was very slightly acid, and the solution filtered hot. The barium sulphate precipitate, which had absorbed much of the humus substance, was not washed. The solution was exactly neutralized with sodium hydroxide, and the resulting fluid, whose volume was 700 cc., was clear and straw-colored and had a bitter-sweet taste. The reactions for barium, calcium, iron, reduced sulphur and tryptophane were negative; phosphate was present and the xantho-proteic reaction was positive. When exposed to the air, bacteria developed with remarkable rapidity. The solution was not toxic to amebas when evaporated to one-half the volume, but evaporation to one-fifth the volume resulted in a solution which was slightly toxic.[2]

This solution of amino acids lacks glycine, which is absent in casein, and tryptophane and cystine, which were destroyed.

In cultivating amebas in this solution it was difficult to prevent the development of bacteria, but a method was devised which served to exclude them almost completely. The amebas were washed five to ten times in sterile water, and the slides upon which they were kept were boiled. The culture solutions were kept in small flasks fitted with capillary tubes. By inverting the flask and warming with the hand, a few drops could be placed upon the slide. After using, the flask was boiled and a test tube placed over its neck. The cultures were examined daily, the amebas washed in sterile water and transferred to new solutions.

The hydrolyzed casein solution proved to be a fairly good medium in which cases of cell division were occasionally observed, but there was

[2] The toxicity of amino acids observed by Burrows and Neymann ('17) upon embryonic chicken cells was probably due to the relatively concentrated solutions used.

never any growth and the changes which preceded death could be seen in about ten days. The addition of glucose resulted in some improvement but it did not prevent death.

DATE	NUMBER OF AMEBAS LIVING	
	Hydrolyzed casein	Hydrolyzed casein + 0.2 per cent glucose
May 1	5	5
May 2	5	6*
May 3	6*	6
May 4	6	6
May 5	6	7
May 6	6	8
May 7	6	8
May 8	Dying	8
May 9	Discontinued	8
May 10		8
May 11		8
May 12		8 Amebas healthy and active
May 13		8 Amebas somewhat abnormal
May 14		7
		Dying. Experiment discontinued

*See footnote to table, p. 265.

DATE	NUMBER OF AMEBAS LIVING
	Hydrolyzed casein + 1 per cent glucose + 0.1 per cent tryptophane + 0.03 per cent cystine + 0.2 per cent milk.
May 23	5
May 24	5
May 25	6*
May 26	6
May 27	6
May 28	6
May 29	6
May 30	6
May 31	6
June 1	6
June 2	6
June 3	5
June 4	3
June 5	3
June 6	1
June 7	0

*See footnote to table p. 265.

Since this death might be due to the absence of some foodstuff, various substances were added to the "diet." The addition of various salts was tried but no improvement was observed. Cystine and tryptophane, so important in the nutrition of higher animals, were added to the solution in concentrations which were shown not to be toxic. A small amount of milk was added to provide the unknown "accessory" food substances, and also certain salts (see lower table on page 268).

No synthetic medium was found for Ameba proteus. The failure may be due to some peculiarity of the cell, such as its enormous size, or to a low permeability.

THE NUTRITION OF THE NON-NUCLEATED CELL

Since, as was shown above, glucose prolongs the life of amebas and apparently acts as a food, it would be interesting to know whether it has a similar effect upon the cell from which the nucleus has been removed. To determine this, the nucleus was removed from a number of amebas, care being taken to remove as little cytoplasm as possible. Under favorable circumstances it is possible to cut the nucleus from the cell without removing more than one-tenth of the cytoplasm. Some of the enucleated amebas were now transferred to water while an equal number of others of equal size were kept in glucose solutions. The usual precautions were taken to prevent the development of bacteria.

DATE	NUMBER OF AMEBAS LIVING	
	Water	0.5 per cent glucose
December 3	9	9
December 4	9	9
December 5	9	9
December 6	9	9
December 7	9	9
December 8	9	9
December 9	9	9
December 10	9	9
December 11	8	8
December 12	4	7
December 13	3	6
December 14	2	4
December 15	0	3
December 16		1
December 17		0
Average life	9.9 days	11.2 days

Although the amebas kept in glucose lived somewhat longer than the controls, the effect was not very striking. Since there are great differences between different amebas, even when selected from the same culture, a somewhat more satisfactory experiment was performed as follows: The nucleus was removed from a number of amebas and then the amebas were divided in half. One-half was placed in water, as a control, while the other half was kept in a solution of glucose.

DATE	NUMBER OF AMEBAS LIVING	
	Water	1 per cent glucose
April 3	12	12
April 4	12	12
April 5	12	12
April 6	12	12
April 7	11	11
April 8	10	11
April 9	9	11
April 10	6	10
April 11	3	6
April 12	2	4
April 13	0	3
April 14		0
Average life	7.4 days	8.7 days

DATE	NUMBER OF AMEBAS LIVING		
	Glucose	Glucose + 0.1 per cent urea	Glucose + 0.01 per cent NH_4NO_3
November 19	10	10	9
November 20	10	10	9
November 21	10	10	9
November 22	10	9	9
November 23	10	9	8
November 24	10	7	8
November 25	10	5	8
November 26	9	2	7
November 27	6	1	5
November 28	5	0	2
November 29	4		2
December 2	0		1
December 3			0
Average life	10.2 days	6.3 days	9.0 days

The non-nucleated cells live longer in glucose solutions and probably use it as a food. This is in agreement with the work of Rous and Turner ('15), who used glucose for the preservation of the non-nucleated red blood corpuscles.

If the life of the non-nucleated cell is prolonged by the use of glucose, is it further prolonged by the use of urea or ammonium nitrate?

The nucleus was removed from twenty-nine amebas, and after washing, ten were placed in glucose, ten in glucose plus urea, and nine in glucose plus ammonium nitrate (see lower table on page 270).

Thus in the non-nucleated cell, the addition of urea to the glucose was not beneficial; on the contrary it was harmful. The addition of the ammonium nitrate was without effect.

Evidence was offered above that the normal, nucleated ameba was able to form some combination between glucose and urea (or some derivative of urea). If it is true that the beneficial effect of glucose plus urea depends upon a synthesis, we may conclude that the non-nucleated cell is unable to perform this synthesis.

RESPIRATION IN THE NON-NUCLEATED CELL

It has been suggested (Loeb, '05) that the nucleus is the organ of oxidation of the living cell and that peculiarities of the non-nucleated cell, such as lack of the power to synthesize or to regenerate lost parts, are the result of the lowered oxidative activity. The non-nucleated pieces, it is said, "die slowly from asphyxia."

If this theory is true, we should expect to find a marked difference between the effect of depriving the non-nucleated cell of oxygen and the effect of depriving the nucleated cell of oxygen. We should expect the cell in which oxidations were occurring most rapidly to be most affected by its removal, whereas the cell which was using very little oxygen should not be greatly affected. According to Child ('15), regions in which respiration is rapid are more susceptible to lack of oxygen and die sooner than regions in which respiration is slower.

The effect of depriving the nucleated and non-nucleated cells of oxygen was investigated in the following manner: Five or more amebas were divided into two pieces of as nearly equal size as possible. After leaving them for a longer or shorter period in air, they were placed in hanging drops, side by side, in an Engelmann gas-chamber. Nitrogen, which had been washed in water, was then passed through the chamber, and this atmosphere was maintained until the death of the organisms.

In the third experiment the nitrogen was first bubbled from a capillary tube through strongly alkaline pyrogallic acid, to remove possible traces of oxygen. In the first experiment, the temperature of the water jacket surrounding the chamber was raised to 26°C. in order to accelerate the experiment. The other experiments were performed at 20°C.

Experiment 1

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
5.00 p.m.	Amebas divided	
9.50 a.m.	Placed in nitrogen	
10.00 a.m.	7	7
11.00 a.m.	7	7
12.00 a.m.	7	6
1.00 p.m.	7	4
1.20 p.m.	7	3
1.30 p.m.	6	1
2.00 p.m.	4	0
2.25 p.m.	3	
2.30 p.m.	2	
2.35 p.m.	1	
2.45 p.m.	0	
Average life	4.4 hours	3.4 hours

Thus there is a distinct difference in the susceptibility to lack of oxygen in the nucleated and non-nucleated cell, but this difference is just the opposite to what we should expect if oxidations were proceeding more rapidly in the nucleated half. Not only does the non-nucleated cell die more quickly when deprived of oxygen, but it is the first to assume a spherical shape and to cease putting out pseudopods.

The opposite experiment, that of increasing the supply of oxygen, is also of interest. Were it true that in removing the nucleus we have removed the organ of oxidation, and that the cell is slowly dying of asphyxia, it should be possible to delay the death of the non-nucleated cell by supplying it with more oxygen. On the contrary, it was found that when the nucleated and non-nucleated halves of amebas were kept in an atmosphere of oxygen, both died in less than twelve hours and the non-nucleated pieces were killed quite as rapidly as those which contained nuclei.

Experiment 2

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
10.30 a.m.	Amebas divided	
11.05 a.m.	Placed in nitrogen	
12.00 a.m.	5	5
1.00 p.m.	5	5
2.00 p.m.	5	5
3.00 p.m.	5	5
4.00 p.m.	5	5
5.00 p.m.	5	5
6.00 p.m.	5	5
7.00 p.m.	5	4
7.30 p.m.	5	2
8.30 p.m.	4	2
9.00 p.m.	4	2
10.00 p.m.	4	1
11.00 p.m.	4	1
12.00 p.m.	4	1
8.00 a.m.	3	1
9.00 a.m.	3	1
9.30 a.m.	3	0
10.00 a.m.	3	
10.15 a.m.	1	
11.00 a.m.	1	
12.00 a.m.	1	
1.00 p.m.	1	
2.00 p.m.	0	
Average life	20.5 hours	11.6 hours

Experiment 3

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
9.00 a.m.	Amebas divided	
10.20 a.m.	Placed in nitrogen	
11.00 a.m.	5	5
12.00 a.m.	5	5
1.00 p.m.	5	5
3.00 p.m.	5	4
4.00 p.m.	5	2
5.00 p.m.	5	2
8.00 p.m.	5	1
9.00 p.m.	4	1
9.30 p.m.	4	0
10.00 p.m.	2	
11.00 p.m.	2	
12.00 p.m.	2	
6.00 a.m.	0	
Average life	14.6 hours	7.4 hours

The experiment was performed in the following manner: Five amebas were cut in half with a fine glass needle, and each half was transferred to a hanging drop in a gas chamber. Oxygen which had been thoroughly washed was now passed through the chamber and the organisms kept under observation until all had disintegrated. The animals were divided at 10 a.m. Oxygen was passed through at 10.30 a.m.

Time of death

NUCLEATED CELL	NON-NUCLEATED CELL
1.30	2.00
2.00	2.10
4.15	3.23
5.00	3.27
6.30	4.15
Average life... 5.4 hours	4.6 hours

The injurious action of oxygen, which is doubtless the result of an increase in the oxidations of the cell, took effect somewhat more rapidly upon the cell which lacked a nucleus. It is difficult to see how a cell could be killed in a few hours by an atmosphere of oxygen if its oxidations have become depressed.

THE EFFECT OF TEMPERATURE UPON THE NON-NUCLEATED CELL

In the experiments hitherto reported, a fairly constant temperature of 20°C. had been maintained. An attempt was now made to determine the effect of temperature upon the nucleated and non-nucleated cells, with the hope of discovering a possible difference in the rate at which chemical changes were occurring in the two cells. Two methods of investigation suggest themselves:

1. The temperature of the medium may be gradually raised until death occurs.

2. The organisms may be kept at a rather high temperature and observed until death occurs.

Both of these methods were used in the order named.

In the first method amebas were placed upon a Pfeiffer warming stage, through which water of any desired temperature could be passed. After dividing the ameba into two equal parts, the temperature of the warming stage was raised at the rate of about one degree Centigrade

per minute. The rate at which the temperature is raised is of importance for by raising the temperature rapidly the organisms may be killed at a comparatively low temperature. As the temperature rises, the amebas soon withdraw all of their pseudopods and assume a spherical shape. This does not, however, indicate the death of the organism for if at this point the temperature is slowly lowered to 20°C. the animals will, in a few hours, send out pseudopods and resume normal movement. If the temperature is raised high enough, the cells will either disintegrate completely or coagulate, with the result that the cell boundary can no longer be seen. The temperature at which the spherical shape was assumed and the temperature at which death occurred are recorded below.

EXPERIMENT	SPHERICAL SHAPE ASSUMED		DEATH	
	Nucleated	Non-nucleated	Nucleated	Non-nucleated
	°C.	°C.	°C.	°C.
1	39	38	51	50
2	40	39	50	50+
3	40	40	47	52
4	39	44	49	48
Average.....	39.5	40.2	49.2	50.1

The effect of gradual cooling was not so easily determined. When the temperature of the fluid was lowered greatly and even under-cooled, there was no injury provided crystallization did not take place. When the solution crystallized, however, as the result of adding a small crystal of ice, the amebas were injured mechanically and disintegrated as soon as the crystals melted.

But the effect of cooling could be shown in a somewhat different manner. The nucleated and non-nucleated parts of an ameba were slowly warmed to 44°C. and then cooled in ten minutes to 22°C. Two minutes later both fragments disintegrated at the same time, the result of the rapid cooling.

Thus no difference could be found in the susceptibility of the nuclear and non-nuclear fragments to a rise or fall of temperature. The other method was now employed: keeping amebas at different temperatures until death occurred.

Eleven amebas were freed from food particles and debris and divided into two equal parts. Both the nuclear and non-nuclear fragments were kept in a moist chamber at an average room temperature of 20°C.

Experiment 1. 20°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 10	11	11
October 11	11	11
October 12	11	11
October 13	8	10
October 14	7	6
October 15, a.m.	7	3
October 15, p.m.	5	2
October 16	4	2
October 17	4	2
October 18	4	2
October 19, a.m.	3	1
October 19, p.m.	1	0
October 20	0	
Average life	6.2 days	5.3 days

Thus, as was shown before, the life of the non-nuclear fragment at ordinary temperature is almost as long as that of the nuclear fragment.

Ten amebas were now divided and each placed in a moist chamber which was kept in a thermostat at 30°C.

Experiment 2. 30°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 22	10	10
October 23, a.m.	10	7
October 23, p.m.	9	7
October 24	9	4
October 25	9	2
October 26, a.m.	6	1
October 26, p.m.	5	0
October 27	3	
October 29	3	
October 30	3	
October 31	1	
November 1	0	
Average life	6.0 days	2.4 days

Apparently the life of the nuclear fragments was not shortened by exposure to high temperature, but this was probably due to the use of healthier amebas in experiment 2. Further experiments showed that the life of nucleated amebas was shortened by raising the temperature but the life of non-nuclear amebas was shortened somewhat more.

Twelve amebas were now divided and placed in a moist chamber at 10°C. The moist chamber was allowed to warm slowly to room temperature before removing the slide for examination.

Experiment 3. 10°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 17	12	12
October 18	12	12
October 19	12	12
October 20	12	12
October 21	12	12
October 22	12	12
October 23	11	11
October 24	11	11
October 25	11	11
October 26	11	11
October 27	11	11
October 29, a.m.	11	9
October 29, p.m.	11	8
October 30, a.m.	11	5
October 30, p.m.	11	4
October 31, a.m.	11	3
October 31, p.m.	10	2
November 1, a.m.	5	0
November 1, p.m.	3	
November 2	0	
Average life	14.5 days	12.8 days

The life of both fragments was prolonged by cold in this case but in two other cases it was distinctly shortened. This may have been due to the use of different cultures.

If susceptibility to high or low temperature may be used as an index to the rate of metabolism, there is no very great difference between the two fragments. There is some evidence that cells in which metabolic processes are rapid show a greater susceptibility to high temperature

than do cells with a low metabolism (Child, '15, p. 66). If this is true, there is some slight indication of a more rapid metabolism in the non-nuclear fragment.

METABOLIC RATE IN THE NON-NUCLEATED CELL

The rate at which metabolic processes are occurring in different parts of an organism or tissue has been very successfully studied by determining their susceptibility to various poisons, especially to potassium cyanide (Child, '13, '15). Child has brought together a great deal of direct and indirect evidence to show that, in general, regions of high metabolism are more susceptible to poisons than are regions of low metabolism. Accordingly, the susceptibility of the nucleated and non-nucleated cell was investigated with a view to determining how the rate of metabolism was affected by the removal of the nucleus.

The amebas were cut in half and one hour after the operation they were transferred to a fresh $\frac{M}{50}$ solution of KCN upon a hollow-ground glass slide. The drop of solution was immediately covered with a cover slip to prevent the escape of HCN. Since this could not be entirely prevented, the solution gradually became weaker and as a result those amebas which were not killed in two or three minutes usually lived for more than an hour. Since small fragments of Ameba were found to be more susceptible than large ones, an effort was made to divide the amebas into equal parts. After more than fifty Amebas had been treated with cyanide, it was obvious that some unknown factor was playing a part in the death of the organisms; for although the non-nucleated fragments usually succumbed first, there were many conspicuous cases in which the nucleated part died much sooner. It seemed possible that the unknown factor might be the difference between the anterior and posterior end. The amebas were divided when they were moving along the bottom in the "limax" condition, and in some cases the non-nuclear fragment was taken from the flowing anterior end while in other cases it was taken from the retracting posterior end. In order to determine whether this made a difference, a number of amebas were divided in such a way that the nucleus remained in the anterior half, while others were so divided that the nucleus remained in the posterior half.

The number of minutes which the nuclear and non-nuclear fragments were able to withstand treatment with cyanide is tabulated below:

Duration of life

NUCLEUS IN ANTERIOR END		NUCLEUS IN POSTERIOR END	
Nucleated	Non-nucleated	Nucleated	Non-nucleated
min.	*min.*	*min.*	*min.*
96	8	190	83
5	34	155	10
45	15	145	5
200	200	85	1
190	190	170	167
45	170	5	5
260	240	145	115
1	2	146	2
290	530	105	2
580	62	100	1
6	180	98	7
2	140	5	3
260	1	1.5	1
260	25	1.9	0.8
300	15	76	2
230	3	74	2
230	140	6	2
320	305	80	11
87	2	80	10
230	6	75	5
185	41	1	0.5
255	450	12	1
450	250	9	3
2	445	25	25
4	100		
305	1		
10	1		
120	1		
1	0.5		
410	410		
330	330		
320	1		
Average: 188.4	134.3	74.6	19.3

When the nucleus was in the anterior end, the non-nuclear fragment died first in 19 cases, the nuclear fragment died first in 7 cases and in the remaining 4 cases each part disintegrated at about the same time. When the nucleus was in the posterior end, however, the nuclear fragment was never the first to disintegrate; the death of both fragments occurred

at the same time in two instances, and in the remaining 22 cases the non-nuclear fragment was the first to disintegate. The complicating factor is now understood. The anterior end of Ameba is more susceptible to cyanide than the posterior end. Essentially the same conclusion was reached by Miss Hyman ('17) in an investigation of the metabolic gradient in Ameba.

When the non-nucleated half is taken from the posterior end, its greater susceptibility to cyanide is not very definite because of the difference which exists between the anterior and posterior parts. When this disturbing factor is removed, however, by cutting the non-nuclear fragment from the anterior end, the non-nuclear fragment is seen to be much more susceptible to cyanide than the nuclear fragment.

The same difference in susceptibility between the nucleated and non-nucleated cells was found when they were kept for twenty hours after the operation before treatment with cyanide.

Whether we can conclude that the rate of metabolism is increased by removal of the nucleus is doubtful. It is possible that removal of the nucleus decreases the resistance to cyanides for some other reason. But, at any rate, the evidence such as it is opposes the view that respiration and metabolism are depressed by removal of the nucleus.

SUMMARY AND CONCLUSIONS

1. An ameba from which the nucleus has been removed may at times exhibit perfectly normal movement; in general, however, movement is somewhat affected by removal of the nucleus.

2. An ameba deprived of its nucleus lives almost as long as an ameba deprived of food.

3. Evidence is offered that Ameba can use glucose in solution as a food. There is also evidence that Ameba can synthesize glucose and urea, or some derivatives of these substances, to form a product which is of nutritive value.

4. Glucose is also of some benefit to the enucleated ameba but the supposed synthesis of glucose and urea can not be performed.

5. The non-nucleated cell is injured more quickly by either a lack or an excess of oxygen than is the normal nucleated cell.

6. The non-nucleated cell is somewhat more susceptible to high or low temperature than the nucleated cell.

7. The non-nucleated cell is more susceptible to cyanide than the nucleated cell.

DISCUSSION

There are two important theories of nuclear function: the theory of *synthesis* and the theory of *oxidation*. According to the former theory, the non-nucleated cell is unable to construct or synthesize new substances; while according to the latter, it is more or less unable to oxidize the substances already present. In reviewing the experiments here reported it is seen that they not only offer strong evidence for the validity of the synthesis theory, but they constitute a practical proof that the oxidation theory is not correct.

From the outset there has been no satisfactory evidence in support of the oxidation theory. It was apparently suggested by some experiments reported by Spitzer ('97). Spitzer obtained from various cells a preparation of nucleoprotein which contained a small amount of iron and which had the property of decomposing hydrogen peroxide. From this it has been concluded not only that all nuclei contain iron, but even that all nucleoproteins contain iron. The latter statement is of course false, and the microchemical evidence in support of the former statement (Macallum, '92) is very unsatisfactory.[3] The presence of iron in the nucleus was taken as an indication that the nucleus played an important rôle in oxidations, but the presence of this iron is open to doubt. Spitzer's catalase may have been derived from the cytoplasm.

The chemical evidence for the oxidation theory is very slight and the physiological evidence is equally so. The assumption that the organ which is furthest removed from the supply of oxygen is the organ of oxidation is not probable in the first place. "It should be borne in mind that oxygen, in order to reach the nucleus, must penetrate a layer of cytoplasm containing reducing substances." (R. S. Lillie, '11.) Moreover, as was shown in the above experiments, the non-nucleated cell may exhibit perfectly normal movements. Since the energy for movement in aerobic organisms is derived from the oxidation of organic matter, it is unlikely that oxidations have become depressed. But if there is a depression of oxidations we should certainly expect (cf. Loeb, '05) to find some improvement in the cell when we increase its supply of oxygen. On the contrary, as has been shown, the cell becomes spherical and dies in a few hours. Also the susceptibility to lack of oxygen,

[3] From a very pure preparation of the sperm heads of the Great Lakes Whitefish, the author was unable to find a trace of iron in 0.5 gram of the dry material by the sulphocyanide test. The ashing was done in the electric muffle and by Neumann digestion, so that there was no question of "masked" iron. (Unpublished.)

the susceptibility to cyanide and the susceptibility to high and low temperature all indicate that respiration and rate of metabolism are quite as rapid in the enucleated cell as in the normal cell.

Recently, however, experiments have been reported which seem to give strong support to this theory; namely, the experiments of Osterhout on the leaf of the Indian Pipe. When the leaf of this plant is cut, the cells which have been injured soon become dark. Osterhout ('17) has shown that this darkening is an oxidation and since it occurs first in the nucleus, he argues that the nucleus is the center of oxidations. These experiments, however, admit of another interpretation. It is reasonable to believe that at the center of the cell there is a strong reducing action and that very little oxygen is present. After the death of the cell, however, oxygen, like other substances, may enter freely, and in the *dying* cell it would be expected that oxidations would be very rapid in the neighborhood of the nucleus, where the reducing action is greatest.

Moreover, Kite and Chambers ('12) found, in cells which were apparently uninjured, that *reduction* was most rapid in the region of the nucleus. The dye janus green becomes red when reduced. Kite and Chambers observed that this reduction occurred first at the centrosome and second in the chromatin of the nucleus.

The experiments here reported offer support to the theory that the nucleus is the organ of synthesis. When a cell is deprived of food the processes of synthesis must, to a great extent, come to a stop, although synthesis of intermediary products of metabolism is still possible. It is probably not a coincidence that a cell deprived of its nucleus lives almost as long as a cell deprived of food.

An opportunity to put the synthesis theory to a direct test was afforded by nutritional experiments. When amebas are kept without food they will die in from one to two weeks, but if glucose is present in solution their life is somewhat prolonged. It appears that the glucose is used as a food. When urea was added the organisms lived even longer and in some cases divided. Urea alone, without glucose, had no such effect, a fact which is interpreted as meaning that the nitrogen compound is combined by the cell with the carbon compound. When nitrogen was already combined with unoxidized carbon, as in the amino-acids, the addition of glucose was unnecessary.

When these experiments were repeated upon the enucleated cell, it was found that glucose was somewhat beneficial and was apparently used as a food. But the further addition of urea was not only of no

benefit, but actually hastened the death of the protoplasm. Apparently non-nuclear protoplasm is unable to combine carbon and nitrogen: the power of synthesis is absent.[4]

There is further evidence of the loss of the power of synthesis in the failure to produce the sticky secretion by means of which Ameba attaches itself to various objects. Normal amebas are firmly attached to the slide and frequently stick to the sides of a pipette when drawn up into it. Non-nucleated amebas, however, shortly after the removal of the nucleus, are but slightly if at all attached to the slide and never stick to the sides of a pipette.

The enucleated cell may move, respire, digest, respond to stimuli and exhibit any activity which is dependent solely upon catabolic or destructive processes of protoplasm. The group of phenomena which it never shows are those of growth and of regeneration and division. The phenomena of growth are essentially phenomena of organic synthesis, and the dependence of growth upon the nucleus implies the dependence of organic synthesis upon the nucleus.

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[4] The configuration of atoms: C-N-C, so characteristic of the nucleic acid molecule, is also the configuration which occurs in the protein molecule at the points where the amino acids are joined. This fact may be not without significance.